The effect of amniotic stem cell transplantation on ovarian function and folliculogenesis in rats with induced ovarian failure

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Abstract

Background:

Premature ovarian failure (POF) occurs in about 1% of female population under the age of 40, leading to menopausal symptoms, many systemic and psychological effects and complications.

Human amniotic membrane-derived stem cells (hAM-MSCs), as a source of stem cells of neonatal birth associated tissues, can differentiate into multiple cell lineages and thus their use for improving the ovarian function emerges as a hope in those patients.

The adipose tissue derived stem cells, as a source of adult stem cells, proved an efficacy on mouse ovary function after chemotherapy-induced ovary failure **(Sun et al., 2013).**

Herein we report the potential use of hAM-MSCs and AD-MSCs to help restore ovarian function in experimental model of chemotherapy induced ovarian failure in rat.

Methods:

Fifty adult female rats were included in the study; 10 remain as a negative control group. The other 40 rats were injected with cyclophasphamide to induce ovarian failure. Two rats were sacrified to confirm ovarian failure. The others (38 rats) were further subdivided randomly into four groups: chemotherapy induced ovarian failure (IOF) through the I.P. route (**IOF** group)**, IOF+PBS** group**, IOF+AM-MSCs** group and **IOF+AD-MSCs** group.All the rats undergo estimation of serum levels of FSH and E2 twice; 15 days and 30 days after injecting the stem cells. Also, 30 days after injecting the stem cells histopathological examination of the ovarian

tissues and gene expressions of Oct-4, Stra8 and integrin beta-1genes were performed.

Results:

IOF group shows decreased follicles and increased interstitial fibrosis with significant decrease of serum E2, significant increased serum FSH level and significant downregulation of Stra8 &integrin beta-1with non-significant decreased expression of oct-4. The same results were demonstrated in the group received PBS. On contrast, in the treated groups, there are increased follicles and corporaewith evident presence of oocytes with significant increased serum E2, significant (in AM-MSCs) or non-significant(in AD-MSCs) decrease in serum FSH levels and upregulation of the three genes with better results in the group receiving AM-MSCs than that received AD-MSCs when compared to the IOF group.

Conclusion:

The current study proved that administration of either hAM-derived MSCs or AD-MSCs exerts a therapeutic effect on the chemotherapy induced ovarian insult in rats improving both hormonal and reproductive functions of the ovary with higher efficacy of hAM-MSCs in that field.

Key words: ovarian failure, chemotherapy, AM-MSCs, AD-MSCs**.**

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List of abbreviations

3β-HSD	3β-hydroxysteroid dehydrogenase
ACTH	Adrenocortical hormone
AD-MSCs	Adipose tissue derived mesenchymal stem cells
AEC	Amniotic epithelial cells
AFC	Antral follicle count
AF-MSC	Amniotic fluid derived mesenchymal stem cells
AKR1C18	Aldo-ketoreductase family 1, member C 18
AMC	Amniotic mesenchymal cells
AMH	Anti-Müllerian hormone
AM-hMSCs	Amniotic Membrane-human Mesenchymal Stromal Cells
AM-MSCs	Amniotic membrane derived mesenchymal stem cells
ANGPT1	Angiopoietin 1
APS	Autoimmune polyglandular syndrome
Bcl-2	B-cell lymphoma 2
BDNF	Brain derived neurotrophic factor
BM-mscs	Bone marrow derived mesenchymal stem cells
BMP15	Bone morphogenetic protein 15
BMT	Bone marrow transplantation
Cables1	Cyclin-dependent kinase-5 and Abl enzyme substrate-1
Cams	Cell-adhesion molecules
CB	Cord blood
CB-mscs	Umbilical cord blood derived mesenchymal stem cells
CCL-12	Chemokine (C-X-C motif) ligand 12
CCL-2	Chemokine (C-C motif) ligand-2
CCR2	Chemokine (C-C motif) receptor 2
CD	Clusters of Differentiation
CDKN2B	Cyclin-dependent kinase inhibitor 2B
CMA	Chromosomal microarray
CM-mscs	Chorionmembrane derived mesenchymal stem cells
CNS	Central nervous system
CTX	Cyclophosphamide
CV-mscs	Chorionic villi derived mesenchymal stem cells
CXCR4	Chemokine (CX- C motif) receptor 4

CYP26B1	Cytochrome P450 family 26 subfamily B polypeptide1
Dcs	Dendritic cells
D-mscs	Decidua derived mesenchymal stem cells
DP-mscs	Dental pulp-MSC
E2	Estradiol
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EPC	Endothelial stem/progenitor cells
ES cells/ ESC	Embryonic stem cells
Fas L	Fas ligand
FGF	Fibroblast growth factor
FISH	Fluorescence in situ hybridization
FM-mscs	Fetal membrane derived mesenchymal stem cells
FMR1 gene	The Fragile X mental retardation 1 gene
FORKO	Follitropinreceptor knockout
FOXL2	Forkhead box L2 gene
FSH	Follicle Stimulating Hormone
FSHR	Follicle-stimulating hormone receptor
GAGS	Glycosaminoglycans
GALT	Galactose—1—phosphate uridylyltransferase
GDF9	Growth differentiation factor 9
GDNF	Glial derived neurotrophic factor
Gnrh	Gonadotropin releasing hormone
GSC	Germlinestem cells
Haec	Human amniotic epithelial cells
HAM	Human amniotic membrane
Hamsc	Human amniotic mesenchymal stromal cells
Hcmsc	Human chorionic mesenchymal stromal cells
Hcmsc	Human chorionic trophoblastic cells
HDAC	Histone deacetylase
HDL	High-density lipoproteins
HGF	Hepatocyte growth factor
HIV	Human Immunodeficiency Virus
HLA-DR	The human leukocyte antigen
HRT	Hormone replacement therapy

HUCPVC	Human umbilical cord perivascular cells
Humenscs	Human menstrual blood stem cells
IBD	Inflammatory bowel disease
ICM	Inner cell mass
Ifnα	Interferon α
IGF-1	Insulin-like growth factor-1
IGF-1	Insulin-like growth factor-1
IL-1	Interleukin-1
IL-1ra	IL-1 receptor antagonist
INHA gene	Inhibin alpha gene
Ips	Induced Pluripotent Stem Cells
IVF	Invitrofertilization
IVF	In Vitro Fertilization
LDL	Low-density lipoproteins
LH	Leutinizing Hormone
LHR	Leutinizing Hormone receptor
MAPK	Mitogen-activated protein kinase
MHC	Major Histocombitability
Mmps	Matrix metalloproteinases
Mscs	Mesenchymal stem cells
NK cells	Natural killer cells
OCT4	Octamer-binding transcription factor 4
PB-MSC	Peripheral bloodderived mesenchymal stem cells
PCNA	Proliferating cell nuclear antigen
PGF	Placental growth factor
PL	Placenta
POD	Premature Ovarian Dysfunction
POF	Premature Ovarian Failure
POI	Primary Ovarian Insufficiency
RA	Retinoic acid
Sca-1	Stem cell antigen-1
SCF	Stem cell factor
SCID	Severe combined immunodeficiency
SDF-1	Stromal-derived-factor-1
SLE	Systemic lupus erythematosus

SSEA4	Stage-specific embryonic antigen-4
STRA8	Stimulated by retinoic acid
TGF	The transforming growth factor
TGF-β	Transforming growth factor beta
Timps	Tissue inhibitor of metalloproteinase
TLR	Toll-like receptor
Tlrs	Toll-like receptors
TNF α	Tumor necrosis factor-α
TRAIL	TNF-related apoptosis-inducing ligand
TSA	Trichostatin-A
TSP-1	Thrombospondin1
UC	Umbilical cord
VCAM-1	Vascular cell adhesion protein 1
VCD	4-vinylcyclohexene diepoxide.
VEGF	Vascular endothelial growth factor
VTE	Venous thromboembolism
WJ-mscs	Wharton's jelly derived mesenchymal stem cells
Zcchc11	Zinc finger, CCHC domain containing 11

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Key words: femoral head, osteonecrosis, methylprednisolone acetate, vitamin E.

Corticosteroid is a risk factor inducing femoral head osteonecrosis. The antioxidant Vitamin E could reduce such osteonecrosis. Sixty adult male albino rats were grouped into: G**I** (normal control), G**II** (Sham control), G**III**-MPSL subdivided into: **(III-A** -3 days) and (**III-B-**3 weeks), G**IV** (MPSL and vitamin E) subdivided into; (**IV-A-** 3 days) and **(IV-B-**3 weeks). **GIII** showed femoral head cortical and medullary pleopathological changes, which were reduced in **GIV** after vitamin E adminstration, being more in subgroup IV-B than IV-A demonstrating a duration dependency.

الكلمات الداله: (راس عظم الفخذ، تنخر العظم، اسيتات ميثيل البريدنيزولون، فيتامين هـ)

تعتبر الستبرويدات من احد العوامل المسببه لتنخر راس عظم الفخذ، و من المحتمل ان يستطيع فيتامين هـ المضاد للاكسده تقلبل هذا التاثبر. و قد تم تقسيم 60 من ذكور الفئران البالغه اللى اربع مجموعات: المجموعه الاولى (الضابطه الطبيعيه)، المجموعه الثانيه (الضابطه الزائفه)، المجموعه الثالثه (اسيتات ميثيل البريدنيزولون) و تم تقسيمها الى مجموعتين فرعيتين (المجموعه الثالثه أ- 3 ايام) و (المجموعه الثالثه ب- 3 أسابيع)، المجموعه الرابعه (، اسيتات ميثيل البريدنيزولون- فيتامين هـ)) و تم تقسيمها الى مجموعتين فرعيتين (المجموعه الرابعه أ- 3 ايام) و (المجموعه الرابعه ب- 3 أسابيع). أظهرت المجموعه الثالثه تغيرات مرضيه فى كل من القشرة و تجويف نخاع العظم و قد تم تقلقل هذا التاثير بعد اعطاء فيتامين هـ فى المجموعه الرابعه و قد اشار ذلك الى التاثر بفترة التعرض.

Introduction

Primary ovarian insufficiency or premature ovarian failure (POI/POF) is one the causes of female infertility. POF is characterized by amenorrhea, hypoestrogenism, and hypergonadotropinism before the age of 40.

There is no proven treatment that restores normal functionality to woman's ovaries **(Kalu and Panay, 2008)**.

A general agreement exists regarding the utility of stem cell therapy for improving ovarian function **(Wang et al., 2013 a).**

Amniotic stem cells are readily available and thus avoid invasive procedures and ethical problems and don't form teratomas when transplanted in vivo **(Klemmt et al., 2011)**. AM derived cells exhibit some embryonic stem cell properties like: expression of pluripotency markers **(Ilancheran et al., 2007)**. High expansion in vitro and multilineage differentiation capacity potential into cells derived from the three germ layers **(Manuelpillai et al., 2011).** Moreover, AM-hMSCs may be considered as superior to adult MSCs in their proliferation and differentiation potential **(Alviano et al., 2007).**

In this study we use - for the first time – hAM-MSCs in rats with chemotherapy induced ovarian failure, comparing them with AD-MSCs, to detect their effect in restoring the ovarian morphology and function.