ADIPOSE DERIVED STEM CELLS ASSISTED LIPOTRANSFER VERSUS ALPHA-LIPOIC ACID THERAPY FOR IMPROVEMENT OF FAT GRAFT SURVIVAL IN ADULT FEMALE ALBINO RAT: Histological study

Thesis

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By:

Aya Essam Omar Ibrahim

Demonstrator of Histology and cell biology
Faculty of Medicine, Ain Shams University

Under Supervision of :

Prof. Dr. Suzi Hanafy Ibrahim

Professor of Histology and cell biology
Faculty of Medicine, Ain shams university

Prof. Dr. Sahar Mohammed Mahdi Omar

Professor of Histology and cell biology
Faculty of Medicine, Ain Shams University

Dr. Gehan Abd El Khalek Ibrahim

Lecturer of Histology and cell biology
Faculty of Medicine, Ain Shams University

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List of Abbreviations

ALA	:	Alpha-Lipoic Acid
ASCs	:	Adipose-derived stem cells
BAT	:	Brown Adipose Tissue
bFGF	:	Basic Fibroblast Growth Factor
BM-MSCs	:	Bone Marrow-derived Mesenchymal Stem Cells
CAL	:	Cell Assisted Lipotransfer
DHLA	:	Dihydrolipoic Acid
DMEM	:	Dulbecco ′s Modified Eagles Medium
ECM	:	Extra Cellular Matrix
EDTA	:	Ethylene diamminetetracetic acid
GSH	:	Glutathione
HGF	:	Hepatocyte Growth Factor
HIF		Hypoxia Inducible factor
INF-ɣ	:	Interferon gamma
LSD	:	Least Significant difference
MAT		Microfragmented Adipose Tissue
MHC-II	:	Major Histocompatibility class II
MSCs	;	Mesenchymal Stem Cells
PBS	:	Phosphate Buffer Saline
PDGF	:	Platelet Derived Growth Factor
PLA cells	:	Processed Lipoaspirate cells
PPARɣ	:	Peroxisome Proliferator-Activated Receptor gamma
RXR	:	Retinoid X Receptor

SEM	:	Standard Error of Mean
SVF	:	Stromal Vascular Fraction
TGF-B	:	Transforming Growth Factor Beta
TNF- α	:	Tumor Necrosis Factor alpha
VEGF	:	Vascular Endothelial Growth Factor
WAT	:	White Adipose Tissue

Abstract

Background and aim of the study: Autologous fat transfer is an ideal method for soft tissue augmentation. This study was conducted to compare between cell-assisted lipotransfer and alpha-lipoic acid therapy in improvement of fat graft survival.

Methods: thirty adult female albino rats were divided into 3 groups. Group I (the control group), Group II (cell assisted lipotransfer), Group III (alpha-lipoic acid). Each group was divided into two subgroups. Subgroups (a) were sacrificed after 7 days and subgroups (b) were sacrificed after 28 days of fat transplantation. Fat grafts were collected from the sites of injection and prepared for histological and immune-histochemical techniques. Morphometrical and statistical analysis were performed.

Results: Histological evaluation of the control group (subgroups a&b) grafts revealed atrophy and death of many adipocytes with formation of numerous fat cysts. Many ghost cells, foam and multinucleated giant cells were detected as well. On the other hand, CAL subgroups (IIa&IIb) showed best results in all histological parameters evaluating graft survival. A significant increase in number of normal sized adipocytes and area % of VEGF reaction together with significant decrease in inflammation and fibrosis compared to subgroups (Ia&Ib) and (IIIa&IIIb) respectively was detected.

While, in alpha-lipoic acid subgroups (a&b), significant increase in number of normal sized adipocytes and VEGF reaction was

detected compared with subgroups (Ia&Ib) respectively. However, insignificant decrease in inflammatory infiltration was observed compared to subgroups (Ia&Ib) respectively.

Conclusion: Both adipose-derived stem cells and alpha-lipoic acid showed improvement in all histological parameters compared with control group. However, enrichment of fat with adipose-derived stem cells showed the best results.

Keywords: Fat transplantation, cell assisted lipotransfer, adipose derived stem cells, alpha-lipoic acid.

Introduction

Autologus fat transplantation is now an ideal filler for augmentation and soft tissue reconstruction in cosmetic and reconstructive surgery **(Toyserkani et al., 2016).** It is now an increasingly attractive method for many procedures including breast reconstruction, facial and hand rejuvenation, treatment of sequelae resulting from radiation therapyand gluteal fat augmentation **(Conde-Green et al., 2013 and Cansancao et al., 2019).**

Autologus fat transplantation is host compatible, readily available, and can be harvested easily and repeatedly as needed without complication arising from allergic or foreign body reactions. The main limitation of this procedure was the low survival rate and high resorption rate of the transplanted fat with graft survival rate ranging from 20 to 90 % **(Jiang et al., 2015).** A recent study by **Choi et al.** found that fat grafting following breast cancer reconstruction retains 45 to 59 percent of its original volume at 49 days and 27 to 54 percent at 140 days. Patients frequently undergo at least two or three sessions of grafting to achieve adequate volume correction **(Gassman et al., 2015).**

Fat graft failure and volume reduction appeared to be related to lack of adequate revascularization within the transplanted fat. Unfortunately, cells die of ischemia before adequate neovascularization does occur, leading to necrosis and early graft loss. Another suggested mechanism of graft volume reduction was adipocyte apoptosis which is related to mechanical and oxidative stresses resulting from fat handling and early ischemia **(Zhu et al., 2010).**

Cell-assisted lipotransfer (CAL) was a method of autologus fat transfer named by **Matsumoto et al.** which was a concomitant transplantation of fat and Adipose-derived Stem Cells (ASCs) **(Matsumoto et al., 2006).** It was supposed that ASCs will increase survival rate of lipotransfer as it can differentiate directly into adipocytes, secrete multiple anti-apoptotic growth factors and promote both angiogenesis and fat regeneration **(Jiang et al., 2015 and Moustaki et al., 2017).**

Alpha-lipoic acid (ALA) is an antioxidant which can act as a potent free radical scavenger and metal chelator, it is a co-enzyme for mitochondrial multi-enzyme complex reactions. ALA has beneficial effects in amelioration of oxidative stress in several tissues. **(Truong et al. 2016)**.

Aim of the work

This study was undertaken to compare CAL and ALA in improvement of fat graft survival.

Review of literature

Adipose tissues

Adipose tissue is a specialized connective tissue that plays an important role in energy homeostasis. Adipose tissue efficiently stores excess energy within lipid dropletsof adipocytes in the form of triglycerides. It also secretes paracrine and endocrine substances and is considered now as a major endocrine organ. Adipocytes modulate energy metabolism and influence general metabolism through secretion of adipocytokines. Adipose tissue is also responsible for the secretion of several proteins into the blood, including leptin, adipsin, resistin, adiponectin, tumor necrosis factor alpha (TNF)-α, and plasminogen-activator inhibitor type 1 (**Lowe et al., 2015**)

Development of adipose tissue:

A mesodermal origin of adipose tissue has been widely accepted. However, the fact that adipose tissue depots appear at different times and possess distinct molecular characteristics suggests that there may be regional heterogeneity among adipose tissue depots. For example, lineage tracing with a marker of neural crest (Sox10) demonstrated that adipocytes in the head and neck are generated from the neuroectoderm, rather than the mesoderm **(Billion et al., 2007).**

There are two types of adipose tissue: unilocular or white adipose tissue (WAT) and multilocular or brown adipose tissue (BAT). In vivo fate-mapping and lineage-tracing studies have established that cells expressing the early myogenic factor Myf5 can give rise to both skeletal myocytes and brown adipocytes in the interscapular and perirenal brown adipose tissue depots **(Timmons et al., 2007)**.

White adipocytes differentiate from perivascular mesenchymal stem cells under the control of peroxisome proliferator-activated receptor gamma (PPARɣ) and the retinoid X receptor (RXR)transcription factors, which are the master regulator for differentiation of committed progenitors into adipocytes in all adipose tissue depots. **(Tang et al, 2008).**

In postnatal life, mesenchymal stem cells that reside in adipose tissue are generally believed to be a principal source for adipocytes during postnatal growth and maintenance of adipose tissue. **(Maumus et al., 2011).**

Mesenchymal stem cells can be found in specialized microenvironment known as *stem cell niche.* The stem cell niche was described as a specific site where stem cells reside for self-renewal and differentiation **(Crane et al., 2017).**

Niches are usually composed of stem cells, supportive stromal cells and extracellular matrix. Cells interact with each other through cell surface receptors, gap junctions and soluble factors. Moreover, blood vessels carry systemic signals and provide a conduit for the recruitment of inflammatory and other circulating cells into the niche. Neural inputs transmit distant physiological cues to the stem cell niche **(Gattazzo et al., 2014)**

Structure of adipose tissue:

It is important to note that the vast majority of cells in adipose tissue are not mature fat cells, but rather are a dynamic mixture of cellular and non-cellular elements, including progenitors, resident/recruited immune cells, fibroblasts, blood vessels, lymphatic vessels, peripheral nerves, and the extracellular matrix (ECM) **(Eto et al., 2009).**

Ultrastructurally, multilocular adipose cells contain huge numbers of mitochondria in addition to lipid vacuoles; this correlates with their function of heat generation through mitochondrial metabolism of fatty acids. The high mitochondrial density is responsible for both the eosinophilia seen histologically and the brown color seen macroscopically, which gives this tissue its alternative name of ‘brown fat’. On the other hand, monolocular adipocytes have prominent smooth endoplasmic reticulum and numerous pinocytotic vesicles, these being involved in lipid biosynthesis and transport. Each cell is surrounded by an external lamina and there is an extracellular matrix composed of reticular fibers (type III collagen) (**Ross et al. 2016**).

Autologous fat transfer and regenerative medicine

Regenerative medicine means using the body's raw materials of repair (cells, matrix, and/or chemical compounds) to restore appearance or function. Regenerative medicine aims to improve the length and quality of patients' life by regenerating, preserving or enhancing the original tissue/organ function. In this context, a variety of novel methods have been considered to address tissue/organ insufficiency, including stem cell-based therapies for the regeneration of damaged tissues and tissue/organ-engineered organs to replace tissue/organ function. Regenerative medicine can be also applied to life-threatening diseases such as myocardial infarction, and in some cosmetic and reconstructive indications. **(Peloso et al., 2018)**

Over a century ago, both fat transplantation and synthetic materials were used as fillers. Common materials such as paraffin, rubber, latex, and silicone were all used starting in the early 1900s as soft-tissue fillers. Recently, more sophisticated biologically compatible fillers have been introduced that are made of hyaluronic acid, such as Hylaform and Restylane. All of these fillers, both synthetic and naturally occurring, have significant limitations that are well known to most surgeons. **(Moseley et al., 2006)**