Effect of Stem Cell Transplantation, Methotrexate combined with Bevacizumab (Anti Vascular Endothelial Growth Factor) on The lung as one of Extra-articular tissues in Adjuvant-Induced Arthritis in Rats: A Pilot Study

Thesis

Submitted in partial fulfillment of the Master Degree of Physical Medicine, Rheumatology and Rehabilitation

By

Youssryea Youssry Mohamed

M.B .B.Ch

Faculty of Medicine, Ain Shams University

Under supervision of

Professor Dr. Nadia Salah Kamel Abd Elbar

Professor of Physical Medicine, Rheumatology and Rehabilitation

Professor Dr. Mona Mahmoud Hussien Arafa

Professor of Physical Medicine, Rheumatology and Rehabilitation

Dr. Mahmoud Mohamed Fathallah

Assistant Professor of Physical Medicine, Rheumatology and Rehabilitation

Faculty of Medicine

Ain Shams University

2014

Acknowledgments

Many thanks will never be enough to express my endless gratitude to **Allah** for giving me the strength and support to carry out this work.

I would like to express my deep appreciation wrapped with great respect to **Prof. Dr. Nadia Salah Kamel Abd-Albar** Professor of Physical Medicine Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University; for her encouragement and expert supervision.

I am greatly honored to express my deepest gratitude to **Prof. Dr. Mona Mahmoud Hussien Arafa**, Professor of Physical Medicine Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University; for her precious advices and valuable observations.

Many great thanks and appreciation are also owed **to Dr. Mahmoud Mohamed Fathallah** Assistant Professor of Physical Medicine Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University; who provided me tireless help and continuous guidance throughout this work.

I am deeply grateful to **Prof. Dr. Hanaa Amer**, Professor of clinical pathology, Faculty of Medicine, Ain Shams University; for her exceptional help and valuable cooperation to accomplish this work.

In deep gratitude I gratefully acknowledge the generous help of **Prof. Dr. Amr Nadim**. Professor of Gynecology and Obstetrics, Faculty of Medicine, Ain Shams University, **Prof. Dr. Fatin Ghazal**. Professor of pathology, Faculty of Medicine, Ain Shams University.

I am indebted to **Ass. Prof. Dr. Somia Ahmed saad-Eldeen** Assistant Professor of pathology, Faculty of Medicine, Ain Shams University, **Dr. Fatma Abd-Al Karim** Fellow of Biochemistry. Medical Research Center, Ain Shams University and **Dr. Ahmed Abd-Allah** Veterinary Fellow, Medical Research Center, Ain Shams University. My gratitude to all of them is limitless. Many great thanks to my partner **Shaimaa Ali** for her great help, sincere cooperation and fruitful support during the conduction of this work.

Lastly, my great thanks to everyone who participated in one way or another in making this work possible.

List of Contents

List of Figures

50	Mild inflammation of the lung parynchyma with focus of lymphocytic infiltration and epithelioid granulomas (H&E x 200)	91
51	Mild Peribronchiolar inflammatory reaction in response to treatment (H&E x 400)	92

List of Tables

List of Graphs

List of Abbreviations

ACD	Acid Citrate Dextrose
ACPA	Anti-citrullinated protein antibodies
ACR	American College of Rheumatology
AIA	Adjuvant-induced arthritis
ANTICCP	Anti- cyclic citrullinated peptides
Anti-MCV	antibodies against mutated citrullinated Vimentin
BM-MSCs	Bone marrow mesenchymal stem cells
BOOP	Bronchiolitis obliterans with organizing pneumonia
BSO	Black seed oil
CD	Cluster of differentiation
CFA	Complete Freund's adjuvant
CIA	Collagen-induced arthritis
CIP	cellular interstitial pneumonia
CM	Centi meter
Combined	Bevacizumab combined with MTX
COPD	Chronic Obstructive Pulmonary airway Disease
COX-	Cyclo-Oxygenase
cPGES	cytosolic PGES
CRP	C-Reactive protein
CT	computed tomography
CTDs	Conictive tissue diseases
CVD	Cardiovascular disease
CXR	Chest X- Ray
DAD	Diffuse alveolar damage
DAS28	Disease Activity Score in 28 Joints
DHFR	dihydrofolate reductase
DIP	Desquamative interstitial pneumonitis
DLCO	Diffusion capacity of the lung for carbon monoxide
DMARDs	Disease-modifying antirheumatic drugs

DNA	Deoxyribonucleic acid
dTMP	deoxythymidine 5'-monophosphate kinase
ELISA	Enzyme-linked immunosorbent assay
ESAF	Endothelial cell-stimulating angiogenesis factor
EULAR	European League Against Rheumatism
FGF	fibroblast growth factor
FLS	Fibroblast-like synoviocyte
FU	fluorouracil
FVC	Forced vital capacity
GI	Gastrointestinal
H & E	Hematoxylin and eosin
HLA	Human leukocytic antigen
HRCT	High-resolution computed tomography
hs	High sensitive
HUCB	Human umbilical cord blood
HUCB-MSCs	Human umbilical cord blood mesenchymal stem cells
IFN	interferon
IG	immunoglobulin
IL	interleukin
ILD	Interstitial lung disease
iNOS	Inducible nitric oxide synthase
IPA	Isopropyl alcohol
IPF	Interstitial pulmonary fibrosis
KRN/NOD mice	K/BxN (or KRN/nonobese diabetic (NOD)) mouse model
LIP	Lymphocytic interstitial pneumonitis
MCP	Metacarpophalengeal
MCP	monocyte chemoattractant protein
MHC	Major histocompatibility complex
μm	Micro meter
mmHg	Millimeter mercury
MNCs	Mononuclear cells
mPGES	microsomal prostaglandin E2 synthase

MRI	Magnetic resonance imaging
mRNA	Messenger-Ribonucleic acid
MSCs	Mesenchymal stem cells
MTX	Methotrexate
NO	Nitric oxide
NSAIDs	Non steroidal anti inflammatory drugs
NSIP	Nonspecific interstitial pneumonitis
PDGF	platelet-derived growth factor
PFTs	Pulmonary function tests
PGES	prostaglandin E2 synthase
PIA	pristane-induced arthritis
PIP	proximal interphalangeal joints
PLA2	phospholipase A2
POCT	point-of-care test
PTPN22	Protein tyrosine phosphatase, non-receptor type 22
RA	Rheumatoid arthritis
RA-ILD	Rheumatoid arthritis associated interstitial lung disease
RDs	Rheumatoic diseases
RF	Rheumatoid factor
RNA	Ribonucleic acid
sPLA2	secretory phospholipase A2
SRAEs	Serious respiratory adverse events
sTNF-R	soluble TNF receptor
TLC	Total lung capacity
TNF	Tumor necrosis factor
UCHMSC	Umbilical cord human mesenchymal stem cells
UIP	Usual interstitial pneumonitis
VEGF	Vascular endothelial growth factor
VILI	Ventilator-induced lung injury

Introduction

Rheumatoid arthritis is a chronic inflammatory disease. Nearly 1% of the population worldwide suffers from RA, with special predation on females of child bearing age **(Carmona et al., 2010)**.

Rheumatoid arthritis affects articular as well as extra-articular organs. vasculopathy plays a crucial role in inflammation associated with the pathogenesis of rheumatoid arthritis with varying degree of tissue damage **(Radic et al., 2013)**.

The pulmonary complications are a significant contributor to excess mortality in patients with rheumatoid arthritis ranking as the second major cause of death in this patient population. While the treatment of rheumatoid articular disease has greatly improved in recent years, as measured by disease activity and quality of life instruments, these benefits have not extended to RA-associated lung disease **(Young et al., 2007)**.

Lungs are a target organ of RA activity pulmonary involvement is one of the extra-articular manifestations of rheumatoid arthritis and includes parenchymal nodules, interstitial involvement, and COPD. Rheumatoid pulmonary vasculitis is rare. Pulmonary disease also may be observed as a toxic event consequent to treatment for RA **(Schneider et al., 2012)**.

Pharmacological Treatments of Rheumatoid arthritis as Disease-modifying antirheumatic drugs slow or halt the progress of the disease and tissue destruction with no effect on regeneration (**Macfarlane et al., 2011)**.

Mesenchymal stem cells are adult stem cells that can be isolated from most adult tissues. Numerous reports on systemic administration of Mesenchymal stem cells leading to functional improvements based on differentiation have been published. Recently, mesenchymal stem cells have been shown to possess immunomodulatory properties **(Yi T et al., 2012)**.

Human umbilical cord mesenchymal stem cells as treatment of adjuvant rheumatoid arthritis in a rat model enhance the efficacy of complete freunds adjuvant-induced arthritis treatment **(Greish et al., 2012)**.

Neoangiogenesis is recognized as a key event in the formation and maintenance of inflammation in rheumatoid arthritis, suggesting that targeting blood vessels in RA may be an effective future therapeutic strategy, VEGF has been demonstrated to have a central involvement in the angiogenic process in RA **(Ewa et al., 2009)**.

Bevacizumab is a "monoclonal antibody" that works by interfering with the process of angiogenesis by targeting and inhibiting human vascular endothelial growth factor. Anti-angiogenic drugs such as bevacizumab may play a significant role in longstanding RA **(Sheikh et al., 2012)**.