A Comparative Study of the Anti-inflammatory and Immunomodulatory Effect of the Anti-Angiogenic Ranibizumab and IL-6R Antagonist Tocilizumab in Rheumatoid Arthritis Model

Thesis
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Dedication

Every challenging work requires self-efforts as well as guidance of olders especially those who were very close to our hearts. I dedicated my humble effort to my sweet & loving **Mother, Brother & Sister** for their emotional & financial support. I confess that without them I couldn't do anything in my life & Because of their unconditional love and prayers, I had the chance to complete this thesis.

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Abstract

Rheumatoid arthritis (RA) is a chronic and progressive autoimmune inflammatory disease associated with irreversible joint destruction that leads to permanent motor disability and compromised quality of life. Although the precise etiology of RA remains obscure, heightened immune response is thought to play a vital role in provoking joint inflammation and bone erosion. This Study aims at evaluating the anti-inflammatory and immuno-modulatory effects of two monoclonal antibodies Ranibizumab (RANI) as anti-VEGF managing agent and Tocilizumab (TCZ) as interleukin-6 receptor (IL-6R) antagonist, against adjuvant induced arthritis in rats. CFA-induced arthritic rats were treated for three consecutive weeks with MTX, TCZ, RANI monotherapy and MTX-TCZ co-therapy. Arthritic score, gait score, ankle diameter, paw thickness, bone erosion markers, inflammatory and angiogenic cytokines and apoptotic markers were determined to assess the anti-arthritic effect. Liver enzymes and histopathological examination of liver and spleen were assessed to evaluate the toxicity profile of the tested therapeutic agents. MTX, TCZ, RANI monotherapy and MTX-TCZ co-therapy significantly enhanced the anti-arthritic parameters in comparison with the CFA-induced arthritic rats. Ranibizumab (RANI) (anti-VEGF managing agent) showed more potent effect than the standard anti-rheumatic drugs Methotrexate (MTX) or Tocilizumab (TCZ). In addition, the combined therapy (MTX-TCZ) proved better efficacy than single agents MTX and TCZ.

Keywords: Adjuvant Induced Arthritis – Rats – Ranibizumab – Tocilizumab – Immune Response.

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List of Abbreviations

ABC	ATP-binding cassette
ACPAs	Anti-citrullinated protein antibodies
Ag	Antigen
ALT	Alanine aminotransferase
AMD	Age-related macular degeneration
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
B-Cell	B lymphocyte
BCl-2	B-cell lymphoma 2
CALA	Collagen antibody- induced arthritis
CD28	Cluster of differentiation 28
CD40	Cluster of differentiation 40
CD40L	Cluster of differentiation 40 Ligand
CD80	Cluster of differentiation 80
CFA	Complete Freund's adjuvants
CIA	Collagen-induced arthritis
COX-II	Cyclooxygenase-II
CRP	C-reactive protein
DC	Dendritic cell
DHFR	Dihydrofolate reductase
DISC	Death- inducing signaling complex
DMARDs	Disease modifying anti-rheumatic Drugs
dTMP	Deoxythymidine monophosthate
dUMP	Deoxyuridine monophosphate
ELISA	Enzyme linked immunosorbent assay

EMEA	The European Medicines Agency's
Fab	Fragment antigen binding
FADD	Fas associated death domain
Fas	First apoptosis signal
FasL	First apoptosis signal ligand
FPGH	Folylpoly glutamate hydrolase
FPGS	Folylpoly glutamyl synthetase
FR	Folate receptor
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
gp-130	Signal transducer glycoprotein 130
H&E	Hematoxylin and eosin
IFA	Incomplete Freund's adjuvant
IFNγ	Interferon gamma
IL-6R	Interleukin-6 receptor
ILs	Interleukins
IL-2	Interleukin-2
IL-17	Interleukin-17
IL-18	Interleukin-18
IL-15	Interleukin-15
IL-1	Interleukin-1
IL-6	Interleukin-6
INN	International Non-Proprietary Name
IP	Intraperitoneal
ITV	Intravitreal
JACK/ STAT	Janus-activated kinase-signal transducer and activator of transcription
JAK	Janus kinase

M-CSF	Macrophage colony-stimulating factor
MHC-II	Major histocompatibility Complex class II molecules
mIL-6R	Membrane bound Il-6 receptor
MMPs	Matrix metalloproteinases
MTX	Methotrexate
MTX-PG	PolyGlutamation of methotrexate
NF-κB	Nuclear factor kappa-B
NSAID	Non-Steroidal anti-inflammatory drugs
OD	Optical density
OPG	Osteoprotegerin
P	Phosphate group (phosphorylation)
P53	Protein 53
PBS	Phosphate buffer saline
PCD	Programmed cell death
PMSF	Phenylmethylsulfonyl fluoride
qPCR	Quantitative polymerase chain reaction
RA	Rheumatoid Arthritis
RANKL	Receptor activator of nuclear factor kappa-B ligand
RANK	Receptor activator of nuclear factor kappa-B
RANI	Ranibizumab
RFC	Reduced folate carrier
RF	Rheumatoid Factor
RT-PCR	Reverse transcription-polymerase chain reaction

SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate- polyacrylamide gel electrophoresis
SEM	Standard error of mean
sIL-6R	Soluble IL-6 receptor
SOCS3	Suppressor of cytokine signaling 3
STAT3	Signal transducer and activator of transcription 3
T-cell	T lymphocyte
TCZ	Tocilizumab
Th1	Lymphocyte T-helper 1cell
Th17	Lymphocyte T-helper 17 cell
TGFβ	Transforming growth factor beta
TLR	Toll Like Receptor
TMYS	Thymidylate synthetase
TNF-α	Tumor necrotic factor- alpha
TRADD	Tumor necrosis factor receptor associated death domain
Treg	Regulatory T cell
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

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