Possible modulatory effect of resveratrol on methotrexate efficacy and pharmacokinetics in a model of experimentally induced arthritis

Thesis presented by

Hend Mahmoud Mohamed Rizk
B.Sc, Ain Shams University (2012)
Demonstrator, Department of pharmacology and toxicology, (2013)
Faculty of pharmacy, Ain shams university.

Submitted to complete the fulfillment of MSc degree in Pharmaceutical Sciences (Pharmacology and Toxicology), Faculty of Pharmacy, Ain Shams University.

Under the supervision of

Prof. Dr. Ebtehal El-Demerdash Zaki

Head of Pharmacology and Toxicology Department
Faculty of pharmacy, Ain shams university

Dr. Reem Nabil Abou El-Naga

Lecturer of Pharmacology and Toxicology, Faculty of pharmacy, Ain Shams University

Faculty of Pharmacy

Ain Shams University

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Hend Rizk

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List of Abbreviations

ACR	*The American College of Rheumatology*
AIA	*Adjuvant induced arthritis*
AICAR	*5-aminoimidazole-4-carboxamide ribonucleotide*
ANOVA	*Analysis of variance*
CFA	*Complete Freund's adjuvant*
CIA	*Collagen induced arthritis*
COX-2	*Cyclooxygenase 2*
CVDs	*Cardiovascular diseases*
DHFR	*Dihydro-folate reductase*
DMARDs	*Disease modifying anti-rheumatic drugs*
DMSO	*Dimethyl sulfoxide*
ELISA	*Enzyme-linked immunosorbent assay*
FLS	*Fibroblast like synoviocytes*
GM-CSF	*Granulocyte-macrophage colony-stimulating factor*
H&E	*Hematoxylin and Eosin*
HLA-DR	*Human Leukocyte Antigen - antigen D Related*
IFN- γ	*Interferon gamma*
Ig	*Immunoglobulins*

IL	*Interleukin*
i.p	*Intraperitoneal*
MMPs	*Matrix metalloproteinases*
MRI	*Magnetic resonance imaging*
MTX	*Methotrexate*
NaOH	*Sodium hydroxide*
NF-κB	*Nuclear factor kappa-light-chain-enhancer of activated B cells*
NK	*Natural killer*
NSAIDs	*Non-Steroidal anti-inflammatory drugs*
OPG	*Osteoprotegerin*
PG	*Prostaglandins*
RA	*Rheumatoid arthritis*
RANKL	*Receptor activator of NF-KB ligand*
RF	*Rheumatoid factor*
RFC_1	*Reduced folate carrier 1*
RSV	*Resveratrol*
s.c	*Subcutaeneous*
SD	*Standard deviation*
TGF-β	*Transforming growth factor beta*
Th17	*T-helper 17 cells*

THF	*Tetrahydro-folate*
TNF-α	*Tumor necrosis factor-alpha*
TRAF	*TNF receptor-associated factor*
VEGF	*Vascular endothelial growth factor*

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Abstract

Abstract

Background: Low dose methotrexate is the cornerstone of rheumatoid arthritis treatment. However, it is seldom used alone and almost usually combined with other anti-rheumatic agents which increases the risk of toxicity. **Objective:** The present study aimed to investigate the modulatory effect of resveratrol on the efficacy of methotrexate, in addition to elucidating the possible mechanisms for this modulatory effect, and also testing the effect of nano-encapsulation of resveratrol on improving this modulatory effect. **Methods:** Complete Freund's adjuvant was used to induce arthritis. Arthritic rats were treated with methotrexate and resveratrol (solution)/resveratrol nano-emulsion. Arthritic score, gait score, ankle diameter, paw volume and histopathology were determined to assess the anti-arthritic effects. Tissue TNF-α, IL-17 levels, and COX-2 activity were evaluated to study the anti-inflammatory effect. While tissue RANKL, and MMP-9 levels were measured to study the effect on bone and cartilage erosion. Finally, tissue caspase-3 activity and cytochrome c level were evaluated to study the effect on apoptosis. **Results:** Combination therapy of resveratrol (50 mg/kg) with methotrexate significantly improved arthritic parameters as compared to methotrexate alone. It also alleviated adjuvant induced inflammation, bone/ cartilage erosion, synovial proliferation, and pannus formation. Moreover, using resveratrol in nano-emulsion form gave the same modulatory effect when given at half the dose administered of the conventional formula. **Conclusion:** resveratrol potentiates the anti-arthritic effects of methotrexate, possibly by acting as anti-inflammatory, and pro-apoptotic. Furthermore, resveratrol nano-encapsulation further improves the modulatory effect of resveratrol on methotrexate efficacy.

Keywords: Resveratrol - Methotrexate - Adjuvant arthritis – nano-emulsion.

Review of Literature

1. Rheumatoid Arthritis

1.1. Background:

Rheumatoid arthritis (RA), is a chronic autoimmune inflammatory disorder which affects the joints and is associated with swelling, stiffness, and pain **(Fishman and Bar-Yehuda, 2010)**. It is usually also accompanied by variable extra-articular manifestations **(Grasasi et al., 1998)**.

There are skeletal remains from North America indicating that the disease existed at least 3000 years ago, and it was given its name by Alfred Baring Garrod in 1859 **(Parish, 1963)**. As the causes of RA are still unknown, cures have not been discovered yet as well. All treatments and therapies which are applied so far are intended largely to reduce symptoms and delay the progress of the disease **(Newman, 1996)**.

Rheumatoid arthritis has been regarded by many as an autoimmune disease whereby the body's immune system attacks its own tissues, based upon the findings of auto-reactivity to collagen type II **(Tarkowski et al., 1989)**, and to non-cartilaginous proteins, for example filaggrin or citrullinated peptides **(Schellekens et al., 1998a)**. A further support for the role of autoimmunity in RA is the presence of rheumatoid factors, commonly regarded as autoantibodies against the Fc region of human IgG. They are present in about 80% of RA patients,

although they are not specific for RA, but occur in many inflammatory disorders, and in symptomless arthritic patients as well **(Ingegnoli et al., 2013)**. The clinical appearance of RA differs so much between individual patients, that one sometimes is tempted to believe that the patients do not have the same disease. **(Arvidson, 2003)**.

1.2. Prevalence:

The prevalence of RA is estimated to be about 1% in most European populations, with a lower prevalence reported for people of Asian and African origin.**(Mody and Cardiel, 2008)**. Prevalence data for RA are now becoming available for many of the developing countries around the world, reporting a prevalence in urban settings that ranges from 0.1% in Algeria **(Slimani and Ladjouze-Rezig, 2014)**, 0.6% in the DRC **(Malemba et al., 2012)**, to an overall prevalence of 2.5% in South Africa **(Solomon et al., 1975)**, and in rural settings that ranges from an overall prevalence of 0.07% in South Africa **(Beighton et al., 1975)**, 0.3% in Egypt **(Usenbo et al., 2015)**, to 0.4% in Lesotho **(Moolenburgh et al., 1986)**.

Females are found to be three times more affected than males **(Anderson et al., 2004)**. Clinically, RA is more prevalent in women before age 50 but is more severe in women after 50 **(Straub, 2007)**. However some small studies from Nigeria, Liberia, and South Africa showed a high male to female ratio that was inconsistent with global findings **(Usenbo *et al.*, 2015)**.