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ORIGINAL RESEARCH

Effect of Peroxisome Proliferator-Activated Receptor Gamma Agonist (Pioglitazone) and Methotrexate on Disease Activity in Rheumatoid Arthritis (Experimental and Clinical Study)

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Abstract

Objective: To investigate the combined effect of both pioglitazone and methotrexate on disease activity of rheumatoid arthritis in a biphasic study; experimental and clinical.

Methods: Experimentally: 50 rats were divided into 5 equal groups; controls, experimental arthritis, methorexate treated (0.1 mg/Kg daily), pioglitazone-treated (10 mg/kg daily), and methotrexate and pioglitazone treated. Clinically: forty-nine diabetic rheumatoid arthritis patients were included. Patients group consisted of 28 patients and they received pioglitazone 30 mg orally beside their usual treatment. Control group consisted of 21 patients and they continued their usual treatment plus placebo. Disease activity was assessed using DAS28 score. Patients were followed up for 3 months.

Results: Pioglitazone produced a significant improvement of serum oxidative stress parameters ($P < 0.05$), and inflammatory cytokines in the treated arthritic group ($P < 0.05$). Clinically, the pioglitazone treated group showed significant improvement in DAS28 ($P = 0.001$) and C-reactive protein ($P < 0.0001$) compared to placebo group.

Conclusion: The concomitant use of the PPAR γ agonist pioglitazone and methotrexate appears to be promising therapeutic strategy for rheumatoid arthritis patients.

Keywords: pioglitazone, PPAR-γ agonist, anti-inflammatory, oxidative stress, rheumatoid arthritis, disease activity

Introduction

Rheumatoid arthritis (RA) is an autoimmune systemic inflammatory disease. Recent studies have found that there is increased prevalence of coronary artery atherosclerosis[1,2] metabolic syndrome[3] and insulin resistance[4] among RA patients. Furthermore, hyperinsulinemia has been associated with disease activity,[4] suggesting that inflammation and hyperinsulinemia somehow interact and facilitate one another.

Pioglitazone is a member of oral antidiabetic agents, the thiazolidinediones (TZDs). TZDs have been proven to improve insulin resistance, reduce circulating hyper-insulinemia and master the regulation of adipogenesis and glucose metabolism.[5,6] They act as ligands for the peroxisome proliferator-activated receptor gamma (PPAR-γ), a member of the nuclear hormone receptor superfamily of transcription factors. PPAR-γ is expressed in a broad array of human tissues,[5] Recently, PPAR-γ has been suggested to be an important immunomodulator; it suppresses the production of inflammatory cytokines[7,8] matrix metalloproteinases,[9] reactive oxygen species[10] and inhibits pro-inflammatory gene expression.[11] Furthermore, PPAR-γ ligands have been shown to induce apoptosis in T lymphocytes,[12,13] and macrophages.[14]

Since the potential anti-inflammatory properties of PPAR-γ ligands on RA activity have been investigated in several experimental arthritis models, this study was conducted to evaluate the effect of PPAR-γ agonist pioglitazone combined with methotrexate (MTX) in RA either experimentally or clinically in RA diabetic patients.

Methods
Experimental study
Drugs used
Methotrexate (Ebewe Co, Austria, ampoule 50 mg/5 ml) and Pioglitazone hydrochloride (Amoun Co, Egypt, tab 30 mg dissolved in 0.5% sodium carboxy methyl cellulose for oral administration).

Animals used
This study was carried on 50 male Sprague-Dawley rats weighing 200–250 gm/rat. Animals had free access to food and water. They were exposed to the same housing conditions of heat and humidity. Animals were handled with the Guide for Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

Animal grouping
Rats were divided into 5 equal groups (10 rats for each). Group 1 Control group: rats received 0.5% sodium carboxy methyl cellulose; Group 2 Control group: Non-treated arthritic group, arthritic rats received 0.5% sodium carboxy methyl cellulose; Group 3 Control group: MTX treated arthritic group, arthritic rats received MTX 0.1 mg/Kg/d;[16] Group 4 Control group: Pioglitazone treated arthritic group, arthritic rats received pioglitazone 10 mg/kg/d;[17] Group 5 Control group: MTX and Pioglitazone treated arthritic group, arthritic rats received MTX and Pioglitazone by the same previous doses. Animals were treated with drugs every 24 h, starting from day 28 orally via gastric tube for 16 days.

Induction of collagen- induced arthritis (CIA)
Collagen-induced arthritis was induced as previously described by Billingham.[15] Bovine type II collagen (CII) was dissolved in 0.01 M acetic acid at a concentration of 2 mg ml^{-1} by stirring overnight at 4 °C. Dissolved CII was frozen at −70 °C until use. Complete Freund's adjuvant (CFA) was prepared by the addition of Mycobacterium tuberculosis H37Ra at a concentration of 2 mg/ml (Difco, Laboratories, Detroit, Michigan). Before injection, CII was emulsified with an equal volume of CFA by centrifugation at 300 r.p.m. for 5 minutes (Virtis 23 homogenizer, Gardmer, New York). On day 1, rats were injected intradermally at the base of the tail with 100 µl of the emulsion (containing 100 µg of CII). On day 21, a second injection of CII in CFA was administered. Animals were received drugs every 24 h, starting from day 28, orally via gastric tube for 16 days.

Arthritic score of CIA
Arthritis was monitored every 4 days by using a macroscopic scoring system ranging from 0 to 4 for each limb, as previously described by Bakharevski et al,[18] as follows. 0, no arthritis; 1, swelling and/or redness of one to two interphalangeal (IP) joints; 2, involvement of three to four IP joints or one larger joint; 3, more than four joints red/swollen; 4, severe arthritis of an entire paw, yielding a score of 0 to 16 per animal.

Biochemical Analyses

After the end of treatment, all rats were sacrified by an overdose of anesthesia. Trunk blood of each rat was collected and allowed to clot and centrifuged at 7000 r.p.m. for 15 minutes. The serum samples were separated carefully and stored at −30 °C until the biochemical assay.

Quantitative determination of serum IL-1β using ELISA kit (Biosource International, Inc, USA).[19] Serum TNF-α was performed by TNF-α ELISA kit (Ray Biotech, Inc, USA).[20] Serum thiobarbituric acid reactive substance (TBARS) was performed using Malondialdehyde Assay kit Northwest, Life Science Specialities, (NWLSS, Canada).[21] Serum total glutathione (GSH) was performed using ELISA kit (Cayman Chemical Company, USA).[22] Serum superoxide dismutase activity (SOD) was performed using an ELISA kit (Cayman Chemical Company, USA).[23]

The absorbance of each sample was read on plate ELISA reader.

Clinical Study
Patients and methods
Inclusion criteria

Forty-nine rheumatoid arthritis (RA) patients with diabetes mellitus type II, attending the Rheumatology and Immunology outpatient clinic in Mansoura University Hospital, Egypt between January 2009 and March 2010 were included in the study. The diagnosis of RA was confirmed by a rheumatologist according to the American College of Rheumatology (ACR) 1987 criteria for RA.[24] Disease activity was assessed by calculating the disease activity for 28 joint indices (DAS28). Online calculator is available at www.das-score.nl.

Exclusion criteria

Pregnant and lactating subjects and patients with history of heart failure, renal failure, active liver disease or elevated aminotransferases greater than twice the upper limit of normal or with known contraindication to pioglitazone were excluded from the study. Patients aged <18 years was not eligible for the current study.

Study design

The present study is a prospective, single blind, therapeutic clinical trial. Eligible participants were RA and diabetic patients. They were subdivided into two groups. The patients group, consisting of the clinically more active (having 2 of the following: >6 swollen joints, >6 tender joints, morning stiffness >one hour) RA patients (n = 28). They received pioglitazone 30 mg orally, once daily for 12 weeks in addition to their treatment. The control group consisted of the remaining 21 patients who continued their treatment plus placebo orally, once daily for 12 weeks. The treatment of rheumatoid arthritis should be stable for at least three months before study entry and should include methotrexate orally 15 mg/week plus non steroidal anti-inflammatory and/or oral corticosteroids ≤7.5 mg/day orally.

All participants underwent two study visits at 0 and 12 weeks for assessment of disease activity, blood sampling, urine pregnancy test and response to therapy. Fasting blood samples were drawn for complete blood count (CBC), erythrocyte sedimentation rate (ESR), C- reactive protein (CRP), liver aminotransferases, and serum creatinine. All subjects also had non study visits at 4, and 8 week for regular follow up and occurrence of side effects.

All subjects gave their informed consent and the study was approved by the Ethical Committee of Mansoura School of Medicine.

Statistical analysis

Data were analyzed using the statistical package SPSS version 17.0. Experimental data were expressed as means ± standard error (SE). Group differences were compared by using ANOVA followed by Tukey test. While clinical data were expressed as means ± standard deviation (SD) and group differences were compared by using t-test. Probability levels <0.05 were considered significant.

Results
Results of the experimental study
characteristics of CIA in rats

There was no macroscopic evidence of either paw erythema or edema in the normal control rats. As shown in Figure 1, animals subjected to CIA showed that the paw erythema and swelling increased in frequency and severity in a time-dependent mode with maximum arthritis indices of approximately 11 observed between 40 to 44 days post-CII immunization. Either MTX, pioglitazone or their combination exerted a significant
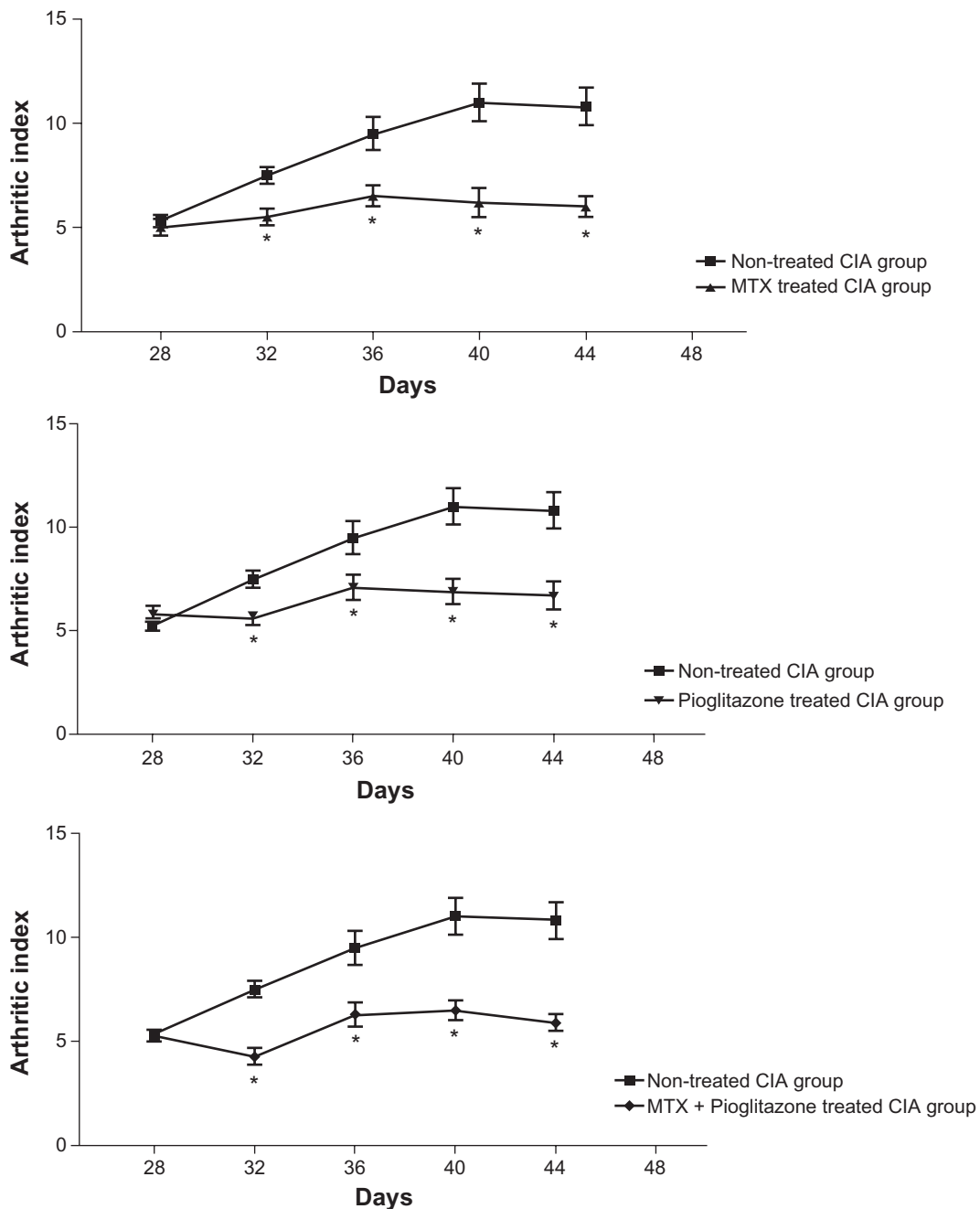
Figure 1. Effect of methotrexate (0.1 mg/kg) and pioglitazone (30 mg/kg) combination on the arthritic index of CIA rats.
Notes: Values are expressed as mean ± SEM. *$P < 0.001$ versus non-tretaed CIA group; Rats number = 10.

suppression ($P1 < 0.001$) of the arthritis index between days 32 and 44 post-CII immunization versus that of non-treated arthritic rats. There were non-significant changes between the combination-treated and the single drug-treated arthritic groups.

Serum oxidative stress parameters

The serum level of oxidative stress parameters were compared to the non-arthritic group. The lipid peroxidation product TRBS was significantly increased (19.14 ± 0.87 versus 8.17 ± 0.41 μ mol/L, $P1 = 0.001$). Meanwhile the antioxidant markers were significantly decreased; serum GSH (6.58 ± 0.33 versus 11.32 ± 0.13 μ mol, $P = 0.001$) and serum SOD (0.138 ± 0.01 versus 0.376 ± 0.02 U/ml, $P = 0.001$).

Either MTX or pioglitazone treatment produced significant decrease of serum TBARS (14.22 ± 1.2 and 14.95 ± 0.97 μ mol/L, $P = 0.001$) and significant

increase of both serum GSH (8.90 ± 0.68, $P = 0.008$ and 7.20 ± 0.59 μ mol, $P = 0.001$) and serum SOD (0.256 ± 0.01, $P = 0.001$ and 0.227 ± 0.02 U/ml, $P = 0.012$) as compared to the non-treated arthritic group.

MTX and pioglitazone combination produced significant decrease of serum TBARS (9.23 ± 0.62 μ mol/L, $P < 0.05$ and significant increase of both serum GSH (10.97 ± 0.76 μ mol, $P < 0.05$) and serum SOD (0.396 ± 0.02 U/ml, $P < 0.05$) as compared to the non-treated, and/or pioglitazone treated arthritic groups (Table 1).

Serum inflammatory mediators

The serum level of the inflammatory mediator, TNF-α was significantly increased in the non treated CIA rats as compared to the non-arthritic group (178.77 ± 14.79 versus 21.71 ± 1.90 pg/ml, $P = 0.001$). Also, IL-1β, was significantly increased in CIA rats versus the non-arthritic group (24.21 ± 2.39 versus 6.46 ± 0.58 pg/ml, $P = 0.001$).

Either MTX or pioglitazone treatment resulted in significant reduction of serum level of TNF-α (45.64 ± 4.05 and 92.79 ± 8.06 pg/ml respectively, $P = 0.001$) and IL1β (12.33 ± 1.42 and 15.43 ± 1.39 pg/ml respectively, $P = 0.001$) in comparison to that of the non-treated arthritic group. It was also noticed that MTX caused significant decrease in both TNF-α ($P = 0.001$) and IL1β ($P = 0.04$) as compared to pioglitazone treatment of the arthritic group.

Further, MTX and pioglitazone combination produced significant improvement of both serum level of TNF-α (36.44 ± 2.53 pg/ml, $P < 0.05$) and IL1β (10.00 ± 0.90 pg/ml, $P < 0.05$) in comparison to the non-treated and pioglitazone treated arthritic group (Table 2). There was a non-significant change between the combination and MTX treated arthritic groups.

Results of the Clinical Study

The pioglitazone treated group of patient showed marked improvement as regard the swollen joint count (SJC), tender joint count (TJC), ESR, CRP, DAS ($P < 0.0001$). This was also associated with significant reduction of blood glucose levels. (Table 3).

Table 1. Effect of methotrexate (0.1 mg/kg) and pioglitazone (30 mg/kg) combination on the serum oxidative stress parameters; TBARS, GSH, SOD, in CIA rats.

	TBARS μ mol/L	GSH μ mol	SOD U/mL
Non-arthritic group	8.17 ± 0.41	11.32 ± 0.13	0.376 ± 0.02
Non-treated CIA group	19.14 ± 0.87 $P1 = 0.001$	6.58 ± 0.33 $P1 = 0.001$	0.138 ± 0.01 $P1 = 0.001$
MTX treated CIA group	14.22 ± 1.20 $P1 = 0.001$ $P2 = 0.001$	8.90 ± 0.68 $P1 = 0.001$ $P2 = 0.008$	0.256 ± 0.01 $P1 = 0.001$ $P2 = 0.001$
Pioglitazone treated CIA group	14.95 ± 0.97 $P1 = 0.001$ $P2 = 0.001$	7.20 ± 0.59 $P1 = 0.03$ $P2 = 0.001$	0.227 ± 0.02 $P1 = 0.001$ $P2 = 0.012$
MTX + Pioglitazone treated CIA group	9.23 ± 0.62 $P1 = 0.04$ $P2 = 0.001$ $P3 = 0.001$ $P4 = 0.001$	10.97 ± 0.76 $P1 = NS$ $P2 = 0.001$ $P3 = 0.45$ $P4 = 0.001$	0.396 ± 0.02 $P1 = NS$ $P2 = 0.001$ $P3 = 0.001$ $P4 = 0.001$

Notes: Values are expressed as mean $\pm$ SEM. $P < 0.05$ was significant. Rats number = 10; *P1* versus non-arthritic group; *P2* versus Non-treated CIA group; *P3* versus MTX treated CIA group; *P4* versus Pioglitazone treated CIA group.
Abbreviations: TBARS, thiobarbituric acid reactive substance; GSH, glutathione; SOD, superoxide dismutase activity; CIA, collagen induced arthritis; MTX, methotrexate.

Table 2. Effect of methotrexate (0.1 mg/kg) and pioglitazone (30 mg/kg) combination on the serum TNF-α, IL-1β in CIA rats.

	TNF-α pg/mL	IL-1β pg/mL
Non-arthritic group	21.71 ± 1.90	6.46 ± 0.58
Non-treated CIA group	178.77 ± 14.79 $P1 = 0.001$	24.21 ± 2.39 $P1 = 0.001$
MTX treated CIA group	45.64 ± 4.05 $P1 = 0.001$ $P2 = 0.001$	12.33 ± 1.42 $P1 = 0.001$ $P2 = 0.001$
Pioglitazone treated group	92.79 ± 8.06 $P1 = 0.001$ $P2 = 0.001$ $P3 = 0.001$	15.43 ± 1.39 $P1 = 0.001$ $P2 = 0.001$ $P3 = 0.04$
MTX + Pioglitazone treated CIA group	36.44 ± 2.53 $P1 = 0.018$ $P2 = 0.001$ $P4 = 0.001$	10.00 ± 0.90 $P1 = 0.015$ $P2 = 0.001$ $P4 = 0.001$

Notes: Values are expressed as mean $\pm$ SEM. $P < 0.05$ was significant. Rats number = 10; *P1* versus non-arthritic group; *P2* versus Non-treated CIA group; *P3* versus MTX treated CIA group; *P4* versus Pioglitazone treated CIA group.
Abbreviations: CIA, collagen induced arthritis; MTX, methotrexate; TNF, tumour necrosis factor; IL, interleukin.

Table 3. Clinical and laboratorial characteristics of pioglitazone and methotrexate treated group of patients before and after treatment by pioglitazone (n = 28).

	Before	After	P
SJC	4.7 ± 1	2.7 ± 0.7	<0.0001
TJC	6 ± 1.2	3.1 ± 1.7	<0.0001
ESR (mm/h)	49.9 ± 11.9	31.4 ± 7.4	<0.0001
DAS28	5.2 ± 0.5	3.8 ± 0.4	<0.0001
CRP (µg/mL)	20.4 ± 4.7	8.1 ± 3.1	<0.0001
Fasting serum glucose (mg/dL)	219.6 ± 57	163.1 ± 28.1	<0.0001
Post prandial serum glucose (mg/dL)	297.1 ± 120.3	217 ± 59.8	0.003

Note: P significant at level <0.05.
Abbreviations: SJC, swollen joint count; TJC, tender joint count; ESR, erythrocyte sedimentation rate; DAS28, disease activity score for 28 joint indices; CRP, C- reactive protein.

On the other hand, the placebo treated (pioglitazone non treated) group of patients showed significant improvement of DAS, TJC, ESR, CRP. However, there were no significant improvement of SJC nor the blood glucose levels (Table 4).

When comparing the pioglitazone treated group of patients with the placebo treated group before treatment, it was found that there were significant differences as regard ESR, DAS28 score indicating that pioglitazone treated patients had more active disease (Table 5).

After 3 months, there were significant reductions of DAS28 and CRP in patients assigned for pioglitazone treatment compared to the other group

Table 4. Clinical and laboratorial characteristics of placebo and methotrexate treated group of patients before and after treatment (n = 21).

	Before	After	P
SJC	4.1 ± 2.5	3.1 ± 1.9	ns
TJC	5.6 ± 2.2	3.6 ± 1.3	0.001
ESR (mm/h)	32.1 ± 8.4	21.7 ± 3.5	<0.0001
DAS28	4.6 ± 0.5	4.2 ± 0.5	0.025
CRP (µg/mL)	18.7 ± 5.2	13.6 ± 3.8	0.001
Fasting serum glucose (mg/dL)	174.4 ± 36.4	163.6 ± 28.8	ns
Post prandial serum glucose (mg/dL)	276.7 ± 113.3	245.7 ± 48.1	ns

Note: P significant at level <0.05.
Abbreviations: ns, non significant; SJC, swollen joint count; TJC, tender joint count; ESR, erythrocyte sedimentation rate; DAS28, disease activity score for 28 joint indices; CRP, C- reactive protein.

Table 5. Clinical and laboratorial characteristics of pioglitazone treated and non treated groups of patients* before treatment by pioglitazone and placebo.

	Pioglitazone treated group	Pioglitazone non treated group	P
Age	43.4 ± 6.8	44.1 ± 7.1	ns
SJC	4.7 ± 1	4.1 ± 2.5	ns
TJC	6 ± 1.2	5.6 ± 2.2	ns
ESR (mm/h)	49.9 ± 11.9	32.1 ± 8.4	<0.0001
DAS28	5.2 ± 0.5	4.6 ± 0.5	<0.0001
CRP (µg/mL)	20.4 ± 4.7	18.7 ± 5.2	ns
Fasting serum glucose (mg/dL)	219.6 ± 57	174.4 ± 36.4	003
Post prandial serum glucose (mg/dL)	297.1 ± 120.3	276.7 ± 113.3	ns

Note: P significant at level <0.05. *all patients received 15 mg methotrexate orally once weekly.
Abbreviations: ns, non significant; SJC, swollen joint count; TJC, tender joint count; ESR, erythrocyte sedimentation rate; DAS28, disease activity score for 28 joint indices; CRP, C- reactive protein.

of patients ($P = 0.001$ and $P < 0.0001$ respectively). Notably, there were no significant differences in blood glucose levels between the two groups (Table 6).

Seven (25%) out of the 28 patients who received pioglitazone in the current study reported weight gain. The increase in weight ranged from 2–4 kg. No other adverse effects were observed.

Table 6. Clinical and laboratorial characteristics of pioglitazone treated and non treated groups of patients* after treatment by pioglitazone and placebo.

	Pioglitazone treated group	Pioglitazone non treated group	P
SJC	2.7 ± 0.7	3.1 ± 1.9	ns
TJC	3.1 ± 1.7	3.6 ± 1.3	ns
ESR (mm/h)	31.4 ± 7.4	21.7 ± 3.5	<0.0001
DAS28	3.8 ± 0.4	4.2 ± 0.5	0.001
CRP (µg/mL)	8.1 ± 3.1	13.6 ± 3.8	<0.0001
Fasting serum glucose (mg/dL)	163.1 ± 28.1	163.6 ± 28.8	ns
Post prandial serum glucose (mg/dL)	217.1 ± 59.8	245.7 ± 48.1	ns

Note: P significant at level <0.05. *all patients received 15 mg methotrexate orally once weekly.
Abbreviations: ns, non significant; SJC, swollen joint count; TJC, tender joint count; ESR, erythrocyte sedimentation rate; DAS28, disease activity score for 28 joint indices; CRP, C- reactive protein.

Discussion

RA is a chronic, systemic inflammatory disease that is characterized by progressive joint destruction. Treatment of rheumatoid arthritis is a major health problem. The available disease modifying antirheumatic drugs (DMARDs) and the recent biologics have promising protecting effects progressive joint destruction.[25,26] The clinical use of these therapies has been limited however due to several issues including safety and cost treatment.

In this study the lipid peroxidation products, TBARS was significantly elevated in the serum of CIA rats. Identification of TBARS in the serum has been recognized as indirect evidence of the effect of free radicals as mediators of tissue damage and inflammatory arthropathy in the pathogenesis of RA.[27] ROS generated by phagocytes in the inflamed rheumatoid joint are not efficiently scavenged resulting in increased levels of lipid peroxidation products both in the synovial fluid and blood as a result of transportation by the circulatory system.[28]

The excessive state of oxidative stress in CIA rats, as evidenced by increased TBARS was associated with impaired antioxidant status components (GSH level and SOD activity) as compared to the control group. This is in agreement with Hassan et al[29] who showed that RA was associated with significant depletion in GSH levels supporting a hypothesis that defense mechanisms against ROS are impaired in RA. In inflammatory arthritis, polymorphonuclear leukocytes and macrophages are responsible for this impaired antioxidant status.[30,31]

The present results showed that MTX treatment caused significant improvement of the arthritis index as well as modulation of the altered parameters produced in CIA rats with respect to serum lipid peroxidation, anti-oxidants levels, serum TNF-α and IL1β as compared to the CIA group. This can be attributed to its established antiinflammatory, anti-proliferative, immunosuppressive effects on activated T lymphocytes, increasing the rate of apoptosis of T cells, increasing endogenous adenosine release, altering the expression of cellular adhesion molecules, influencing production of cytokines, humoral responses and bone formation.[4,32,33]

In this study, it has been demonstrated that pioglitazone produced a significant improvement of the arthritis index that was associated with a significant reduction of the oxidative stress markers and the serum cytokines (TNF-α and IL1β) as compared to CIA group.

MTX and pioglitazone combination also exerted a significant improvement of the arthritis index and significant reduction of the oxidative stress markers and the serum cytokines (TNF-α and IL1β) as compared to the non-treated CIA group.

Tomita and associates[34] observed that oral administration of PPAR-γ agonist resulted in reduced concentrations of proinflammatory cytokines at both the local and systemic levels. PPAR-γ agonist also decreased the expression of IL-1β and TNF-α in arthritic synovium in joints of mice with established collagen-induced arthritis. In support of this proposal the finding of Koufany et al[35] that the TZDs (rosiglitazone 10 mg/kg/day or pioglitazone 30 mg/kg/day) decreased the expression of IL-1β and TNF-α in inflamed synovium, which is a primary source for systemic inflammatory cytokines. This could be due to the ability of PPAR-γ agonists to inhibit IL-1β induced nitric oxide synthase expression and synthesis in chondrocytes from patients with osteoarthritis. PPAR-γ ligands also inhibited IL-1β induced metalloproteinase-13 (MMP-13) expression and production in chondrocytes. In addition to IL-1β, other cytokines produced in arthritic joint tissues, such as IL-17 and TNF-α, may contribute to joint destruction through production of nitric oxide (NO) and MMP-13. As expected, PPAR-γ activators inhibited the production of NO and MMP-13 in response to TNF-α, IL-17, suggesting that PPAR-γ activators may target common pathways leading to NO and MMP-13 production.[8] Further, PPAR-γ activator inhibits IL-1β induced COX-2 expression and PGE$_2$ production by human chondrocytes and synovial fibroblasts.[36] A high level of COX-2 induced prostaglandins is known to be associated with the oxidative stress as COX-2 over expression induces the generation of free radicals and lipid peroxide formations.[37] Consequently, modulation of COX-2 by PPAR-γ agonist is another mechanism of the anti-inflammatory, antioxidative effect of pioglitazone. Moreover, the anti-arthritic potency of PPAR-γ agonists may be due to their ability to inhibit the NF κB pathway in arthritic tissues.[38] Also, TZDs decreased the expression of basic fibroblast growth factor (bFGF), which is a

powerful mitogen for both synovial fibroblasts and endothelial cells whose neutralization was reported to attenuate arthritis severity.[39]

To the best of our knowledge, this is the first clinical study evaluating the effects of a PPAR-γ agonist (pioglitazone) combined with MTX as therapy for rheumatoid arthritis. In the present study, the group of patients treated with pioglitazone exhibited significant improvement either clinically or in the laboratory. Also, there were significant reductions in the ESR, CRP and DAS28 compared to the placebo treated group of patients. Of note, there was no difference in the blood glucose level between the two groups at the end of the study, indicating that the improvement in disease activity indices and markers is not due to the antibiabetic effect of pioglitazone. These results could be explained by the suggested anti-inflammatory effects of PPAR-γ ligands.[40] Recent studies have indicated that many of the cells involved with RA, namely mononuclear leukocytes,[41] articular cartilage and chondrocytes[42] express PPAR-γ receptors.

In the experimental phase of the study, we have highlighted the participation of cytokines in the inflammation, even in the early phases of induced arthritis. It was reported that PPAR-γ agonists can inhibit the expression of adhesion molecules,[43] the release of TNFα from the cultered RA synovial membrane cells and spleen cells.[34,44]

Thus, there are several potential mechanisms by which PPAR-γ agonists could exert their anti-inflammatory effect.

Lately, the PPAR-γ agonists are emerging potential therapies for inflammatory cardiovascular diseases,[11] psoriatic arthritis,[45] multiple sclerosis,[40] chronic obstructive pulmonary disease[46] and glomerulosclerosis.[47] The findings in the present study suggest that PPAR-γ agonist (pioglitazone) could be a potential therapeutic agent for RA.

Conclusion

In conclusion, this study demonstrated that the concomitant use of MTX and pioglitazone may cause a synergistic effect in rheumatoid arthritis. We speculated that the observed beneficial effects of both drugs may be dependent upon a combination of the following pharmacological actions; 1) They inhibit inflammatory cytokines TNF-α, IL-1β. 2) They

appear to prevent the activation of ROS, ultimately the degree of tissue injury. The antioxidant and the anti-inflammatory effects of pioglitazone and MTX support the possibility that patients with RA may benefit from therapy with both of the above in combination.

Abbreviations

RA, Rheumatoid arthritis; TZDs, thiazolidinediones; PPAR-γ, the peroxisome proliferator-activated receptor gamma; MTX, methotrexate; CIA, collagen II induced arthritis; DAS28, disease activity for 28 joint indices; CBC, complete blood count; ESR, erythrocyte sedimentation rate; CRP, C- reactive protein; TBARS, thiobarbituric acid reactive substance; GSH, glutathione; SOD, superoxide dismutase activity; TNF, tumour necrosis factor; IL, interleukin; SJC, swollen joint count; TJC, tender joint count.

Authors Contributions

All the authors shared study design, data collection and literature research. DS designed, performed and wrote the initial version of the clinical study and critically revised the final version of the manuscript and she was responsible for the statistical analysis of the clinical results. EET designed, conducted and wrote the initial version of the study. HAM and VB designed and performed the experimental study and were responsible for the statistical analysis of the experimental part and writing the initial version of the manuscript. DS and AZE were responsible for performing the biochemical analyses and helped in writing the initial version of the manuscript.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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