CLINICAL STUDY

Treatment with the PPARγ agonist rosiglitazone downregulates interleukin-1 receptor antagonist in individuals with metabolic syndrome

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Abstract

Objectives: Thiazolidinediones (TZDs) reduce insulin resistance, but also have pleiotropic properties including effects on inflammation. The balance between protective and proatherogenic effects may differ in various patient populations. We studied the effect of rosiglitazone on inflammatory markers in patients with metabolic syndrome (MetSyn).

Methods: In a cross-over randomized controlled trial, 23 subjects with MetSyn were assigned to treatment with rosiglitazone that was uptitrated from 4 mg/day for 6 weeks followed by 8 mg/day for 6 weeks or matching placebo for 12 weeks, and then to the opposite treatment for 12 weeks. Plasma levels of inflammatory and metabolic markers were measured during follow-up.

Results: Our main findings were i) compared to placebo, rosiglitazone significantly decreased the plasma levels of the naturally occurring interleukin (IL)1 inhibitor, IL1 receptor antagonist (IL1Ra; \( P<0.001 \)), potentially reflecting inflammatory effects on the IL1 system; ii) parallel to this, rosiglitazone decreased plasma levels of IL10 (\( P=0.029 \)) further suggesting inflammatory effects; iii) rosiglitazone decreased uric acid levels (\( P=0.001 \)), and monocyte chemoattractant protein-1 (\( P=0.05 \)) and C-reactive protein (\( P=0.06 \)) tended to be lower after rosiglitazone than placebo, suggesting potential pro- and anti-inflammatory effects simultaneously and iv) in vitro, rosiglitazone enhanced IL1Ra and decreased IL1 in THP-1 monocytes, illustrating the complex effects of these medications, potentially exhibiting anti-inflammatory effects on the IL1 system in certain tissues or cells at least at certain concentrations.

Conclusion: Our findings suggest inflammatory effects on the IL1 system during rosiglitazone therapy in MetSyn. However, anti-inflammatory effects were also observed, and the net effect of TZDs in MetSyn should be further investigated.

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Introduction

Patients with metabolic syndrome (MetSyn) have a high risk of developing cardiovascular morbidity and mortality (1). Each of the risk factors comprising this syndrome interacts synergistically, causing or accelerating the progression of atherosclerosis. Thus, although it is not known whether insulin resistance (IR) universally precedes the development of MetSyn, this syndrome is a precursor of type 2 diabetes. However, the classical risk factors may not alone account for the increased risk of cardiovascular disease in MetSyn. In the last decade, experimental and clinical data have illuminated a role of inflammation in atherogenesis (2), and this concept could also be applied to patients with MetSyn. Thus, several studies have shown raised serum levels of inflammatory markers such as C-reactive protein (CRP) (3), interleukin (IL)6 (4), and tumor necrosis factor α (TNFα) (5) in these patients. In fact, it has been suggested that the MetSyn, type 2 diabetes, and atherosclerotic disorders are multifactorial diseases, which are all characterized by chronic inflammation (1, 6).

Thiazolidinediones (TZDs) are a class of drugs that reduce IR and thereby are used in clinical practice to treat patients with type 2 diabetes (7). TZDs are selective and potent agonists for the peroxisome proliferator-activated receptor (PPAR)γ, and influence the transcription of genes that regulate lipid and glucose metabolism (8). Recent data suggest that PPARγ agonists also may
modulate other biological processes that are involved in atherosclerosis, including inflammation (9, 10). For example, the PPARγ agonist rosiglitazone reduced serum concentrations of CRP in patients with type 2 diabetes and in patients with coronary artery disease (CAD) without diabetes (11). Some of the anti-inflammatory effects of TZDs may be secondary to their metabolic effects, but could also, at least in part, be a direct effect of PPARγ activation. However, the effect of PPARγ agonists on inflammation is still unclear, and few studies are available regarding the potential anti-inflammatory effects of PPARγ agonists in MetSyn prior to the development of diabetes. On the other hand, recent studies have suggested that PPARγ agonists have other effects that may exacerbate CAD (12).

The purpose of this study was to elucidate the effect of the PPARγ agonist rosiglitazone on inflammatory responses in patients with MetSyn. In a placebo-controlled, cross-over study, we compared the effects of rosiglitazone treatment for 12 weeks with placebo on a wide range of inflammatory markers in patient with MetSyn, who had not yet developed diabetes.

Materials and methods

Subjects
Subjects were recruited from the patient charts at the Department of Preventive Cardiology at Ullevål Hospital, Oslo University Hospital, Oslo, Norway and by newspaper advertisement, and were screened for enrollment in the study in November and December 2004. Eligible subjects were nonsmoking men (30–75 years) and women (45–75 years, at least 1 year postmenopausal or oophorectomized). All individuals had two or more of the following National Cholesterol Education Program (NCEP) 2001 criteria for MetSyn in addition to body mass index (BMI) > 30 kg/m²: i) waist > 102 cm (men) or > 88 cm (women); ii) fasting triglycerides > 1.69 mmol/l; iii) high-density lipoprotein (HDL) cholesterol < 1.03 mmol/l (men) or < 1.29 mmol/l (women); iv) blood pressure ≥130/≥85 mmHg; and v) fasting plasma glucose ≥ 6.1 mmol/l. Subjects were not included if they i) had taken statins, anti-hypertensives, anti-diabetes drugs, or hormone substitution (women); ii) had any known autoimmune or chronic inflammatory disease or active infection; iii) had any known cardiovascular disease, including CAD and heart failure; iv) were at need for statins, anti-hypertensives, or anti-diabetic drugs according to guidelines or physicians judgment; v) had fasting plasma glucose > 8 mmol/l, fasting triglycerides > 7.0 mmol/l, systolic blood pressure ≥ 160 mmHg, or diastolic ≥ 100 mmHg; vi) had used omega-3 fatty acid preparations within 2 weeks of screening; vii) were abusing alcohol or narcotics; viii) had any unstable medical or psychiatric disease; ix) had hemoglobin concentrations below the reference level at screening; x) had aspartate aminotransferase or alanine aminotransferase > 2 × upper reference level at screening; and xi) had any liver or kidney disease; or xii) had BMI > 40 kg/m².

Rosiglitazone and placebo tablets
Visually matched tablets containing either rosiglitazone (Avandia, 4 and 8 mg) or placebo were provided by GlaxoSmithKline.

Study design
Following screening, subjects were randomized to treatment with rosiglitazone that was uptitrated from 4 mg/day for 6 weeks followed by 8 mg/day for 6 weeks or matching placebo tablets for 12 weeks, and then to the opposite treatment (placebo or rosiglitazone) for 12 weeks for a total of 24 weeks. The patients were instructed to keep their diet and body weight stable during the study. Blood sampling and clinical evaluations were performed at the day of screening, randomization (baseline), after 12 weeks and at 24 weeks at the end of the study. The study was approved by the regional ethical committee and the Norwegian Medicines Agency (Eudract number 2004-001026-24) and conformed to the Declaration of Helsinki. Written informed consent was obtained from each patient.

Blood sampling protocol
Blood samples were drawn between 0800 and 1100 h after an overnight fast into pyrogen-free EDTA tubes (plasma) or tubes without any additives (serum), immediately immersed in melting ice, and centrifuged within 30 min at 2000 g for 20 min (platelet-poor plasma) or after coagulation (serum). Serum and plasma were stored at −80 °C until analysis, and samples were thawed only once.

Cell culture
The human monocytic cell line THP-1 (American Type Culture Collection, Rockville, MD, USA) was cultured for 4 days in RPMI 1640 (PAA Laboratories, Pasching, Austria) supplemented with 2.5% fetal bovine serum (Gibco) in the presence of recombinant human TNFα (5 ng/ml, R&D Systems, Minneapolis, MN, USA) before further incubation with or without different concentrations of the PPAR-agonist rosiglitazone (Cayman Chemical, Ann Arbor, MI, USA). Cell pellets and cell-free supernatants were stored at indicated time points at −70 °C until further analysis. Endotoxin levels in media and rosiglitazone were < 10 pg/ml (limulus amoebocyte lysate test; BioWhittaker, Walkersville, MD, USA).
**Biochemical and immunological analysis**

Routine clinical chemistry, including leukocyte and leukocyte subset counts, was performed as previously reported (13, 14). Serum glucose was analyzed by the glucose hexokinase method, and HbA1c was analyzed by colorimetric and immunoturbidimetric methods in whole blood (Cobas Integra System, Roche Diagnostics GmbH). Serum levels of insulin were measured by a RIA method (Insulin Coat-A-Count, DPC, Los Angeles, CA, USA). IR and pancreatic B-cell function were calculated by the homeostasis model assessment (HOMA-IR and HOMA-B respectively) method (15). Total cholesterol, low-density lipoprotein (LDL) and HDL cholesterol, and triglycerides were measured using enzymatic colorimetric tests (Hitachi 917; Roche Diagnostics). CRP was measured in serum using a high-sensitivity particle-enhanced immunoturbidimetric assay on a modular platform (Roche Diagnostics). Plasma levels of IL1β, IL6, IL10, monocyte chemoattractant protein (MCP)1, and TNFz were determined by using a multiplex cytokine immunoassay from Bio-Rad Laboratories, and were further analyzed on a Multiplex Analyser (Bio-Rad Laboratories). Plasma levels of neopterin and soluble CD40 ligand (sCD40L) were measured by enzyme immunoassays (EIAs) provided by Brahms (Henninsdorf, Germany) and Bender MedSystems (Vienna, Austria) respectively. Plasma levels of osteoprotegerin, IL1 receptor antagonist (IL1Ra), CC chemokine ligand 21 (CCL21), and soluble TNF receptor type 1 (sTNF-R1) were measured by EIAs obtained from R&D systems. Plasma levels of von Willebrand factor were determined by EIA as reported elsewhere (16). Concentrations of IL1β and IL1Ra in monocyte supernatants were analyzed by EIAs (R&D Systems). The intra- and inter-assay coefficient of variation was <10% for all assays.

**Statistical analysis**

Data are presented as means with s.d. with the exception of CRP for which median and interquartile range values are shown. Screening characteristics of the included patients at baseline and blood test parameters were compared using Student’s t-test. Analysis for the presence of period or carry-over effects revealed no significant effects (data not shown). Thus, at the end of each period (rosiglitazone or placebo), results were combined for both groups and shown for all participants. The estimated treatment effects (values after rosiglitazone compared with values after placebo) were analyzed with paired t-test for laboratory parameters and Wilcoxon signed rank test for inflammatory profile including CRP (because of a skewed distribution as estimated by the Kolmogorov–Smirnov test). Throughout, we report two-tailed P values, and values <0.05 were considered significant. However, particular attention should be directed toward smaller P values <0.01, because a considerable number of P values have been calculated.

**Results**

**Subject disposition and characteristics**

Twenty-six subjects were screened for inclusion in the study. One potential subject was not randomized because he was diagnosed with chronic lymphatic leukemia at screening. One male subject withdrew on the visit after randomization because of problems with venipuncture. One female subject stopped taking study medication (placebo) due to diarrhea and withdrew from the study. This left 11 female and 12 male participants.

At baseline, 12 subjects were assigned to receive rosiglitazone first, and 11 subjects were assigned to receive placebo first and each group switched to the opposite condition after 12 weeks. Baseline characteristics were similar in both groups with the exception of HDL cholesterol, which was higher in the group that started on placebo (Table 1). During the study period, there were no significant differences in changes in body weight, BMI, waist circumference, heart rate, or systolic and diastolic blood pressure between the two groups (data not shown; P > 0.15 for all).

**Metabolic effects**

Total cholesterol increased significantly on rosiglitazone versus placebo, primarily reflecting an increase in LDL

**Table 1** Screening characteristics of the study subjects. Data are given as mean (s.d).

<table>
<thead>
<tr>
<th>Rosiglitazone first (n=12)</th>
<th>Placebo first (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic and clinical characteristics</td>
<td></td>
</tr>
<tr>
<td>Males/females</td>
<td>7/5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.2 (6.6)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>32.0 (4.5)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>104 (7)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>135 (11)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>90 (4)</td>
</tr>
<tr>
<td>Heart rate/min</td>
<td>61 (3)</td>
</tr>
<tr>
<td>Fasting laboratory parameters</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.2 (0.9)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2 (0.1)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.6 (0.9)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.7 (0.6)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.3 (0.6)</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>65 (31)</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>3.9 (3.7)</td>
</tr>
<tr>
<td>Metabolic syndrome components (numbers)</td>
<td>3.0 (0.7)</td>
</tr>
</tbody>
</table>

*P<0.006.
*All individuals had two or more of the criteria for MetSyn (National Cholesterol Education Program, 2001) in addition to body mass index > 25 kg/m² (see Material and methods).
cholesterol (Table 2). The apolipoprotein B/A1 ratio was also significantly higher on rosiglitazone versus placebo. As for the other lipid parameters, there were no significant differences between rosiglitazone and placebo periods (Table 2). Whereas there were no differences in fasting glucose levels, fasting insulin concentrations were lower after rosiglitazone treatment than after placebo treatment (Table 2). HbA1c concentration was higher after the rosiglitazone period versus the placebo period (Table 2), but all individuals had HbA1c levels within normal limits during the study period. Moreover, after rosiglitazone therapy, there was a decrease in IR (HOMA-IR) accompanied by a decrease in insulin release or pancreatic B-cell function (HOMA-B), potentially reflecting improved insulin sensitivity during rosiglitazone therapy (Table 2).

### Table 2

Laboratory parameters at baseline and at the end of each treatment period (n=23). Mean (s.d.) values are shown.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Rosiglitazone</th>
<th>Placebo</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipids and lipoproteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.40 (0.93)</td>
<td>6.13 (0.9)</td>
<td>5.40 (0.73)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.34 (0.41)</td>
<td>1.38 (0.38)</td>
<td>1.31 (0.32)</td>
<td>0.1</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.51 (0.81)</td>
<td>4.07 (0.90)</td>
<td>3.56 (0.74)</td>
<td>0.003</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.68 (0.84)</td>
<td>1.85 (1.15)</td>
<td>1.73 (1.01)</td>
<td>0.6</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>1.01 (0.21)</td>
<td>1.00 (0.22)</td>
<td>0.94 (0.17)</td>
<td>0.08</td>
</tr>
<tr>
<td>Apolipoprotein A1 (g/l)</td>
<td>1.36 (0.25)</td>
<td>1.27 (0.26)</td>
<td>1.34 (0.21)</td>
<td>0.08</td>
</tr>
<tr>
<td>Apolipoprotein B/A1</td>
<td>0.77 (0.21)</td>
<td>0.84 (0.33)</td>
<td>0.72 (0.18)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Carbohydrate metabolism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.20 (0.51)</td>
<td>5.19 (0.51)</td>
<td>5.31 (0.57)</td>
<td>0.3</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>72 (45)</td>
<td>45 (23)</td>
<td>59 (31)</td>
<td>0.01</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>5.65 (0.28)</td>
<td>5.74 (0.27)</td>
<td>5.60 (0.27)</td>
<td>0.02</td>
</tr>
<tr>
<td>HOMA B (%)</td>
<td>105 (37)</td>
<td>75 (24)</td>
<td>86 (32)</td>
<td>0.04</td>
</tr>
<tr>
<td>HOMA IR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.39 (0.87)</td>
<td>0.84 (0.46)</td>
<td>1.12 (0.59)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Clinical chemistry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uric acid (μmol/l)</td>
<td>372 (65)</td>
<td>365 (89)</td>
<td>408 (82)</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>72 (14)</td>
<td>72 (15)</td>
<td>70 (13)</td>
<td>0.02</td>
</tr>
<tr>
<td>ASAT (U/l)</td>
<td>26 (7)</td>
<td>24 (6)</td>
<td>28 (8)</td>
<td>0.04</td>
</tr>
<tr>
<td>ALAT (U/l)</td>
<td>35 (18)</td>
<td>27 (11)</td>
<td>35 (17)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Hematology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.5 (0.8)</td>
<td>13.7 (1.1)</td>
<td>14.5 (0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Red blood cells (10&lt;sup&gt;12&lt;/sup&gt;/l)</td>
<td>4.85 (0.30)</td>
<td>4.63 (0.40)</td>
<td>4.84 (0.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelets (10&lt;sup&gt;9&lt;/sup&gt;/l)</td>
<td>263 (59)</td>
<td>238 (51)</td>
<td>246 (51)</td>
<td>0.3</td>
</tr>
<tr>
<td>White blood cells (10&lt;sup&gt;9&lt;/sup&gt;/l)</td>
<td>5.98 (1.48)</td>
<td>5.37 (1.19)</td>
<td>5.91 (1.28)</td>
<td>0.003</td>
</tr>
<tr>
<td>Neutrophils (10&lt;sup&gt;9&lt;/sup&gt;/l)</td>
<td>3.34 (1.13)</td>
<td>2.77 (0.92)</td>
<td>3.20 (1.05)</td>
<td>0.01</td>
</tr>
<tr>
<td>Lymphocytes (10&lt;sup&gt;9&lt;/sup&gt;/l)</td>
<td>1.90 (0.54)</td>
<td>1.88 (0.49)</td>
<td>1.98 (0.53)</td>
<td>0.08</td>
</tr>
<tr>
<td>Monocytes (10&lt;sup&gt;9&lt;/sup&gt;/l)</td>
<td>0.48 (0.16)</td>
<td>0.47 (0.16)</td>
<td>0.48 (0.14)</td>
<td>0.6</td>
</tr>
<tr>
<td>Eosinophils (10&lt;sup&gt;9&lt;/sup&gt;/l)</td>
<td>0.19 (0.09)</td>
<td>0.18 (0.09)</td>
<td>0.19 (0.10)</td>
<td>0.5</td>
</tr>
<tr>
<td>Basophils (10&lt;sup&gt;9&lt;/sup&gt;/l)</td>
<td>0.10 (0.11)</td>
<td>0.06 (0.04)</td>
<td>0.06 (0.03)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Paired t-tests.

<sup>b</sup> Insulin resistance and pancreatic B-cell function were calculated by the homeostasis model assessment (HOMA-IR and HOMA-B respectively) method.

### Table 3

Inflammatory profile at baseline and at the end of each treatment period (n=23). Data are given as mean (s.d.) (normally distributed data) except for C-reactive protein (CRP), interleukin (IL1b), and ILRa for which median and 25 and 75th percentiles are shown (not normally distributed data).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Rosiglitazone</th>
<th>Placebo</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/l)</td>
<td>3.0 (1.6, 5.1)</td>
<td>1.6 (1.1, 2.6)</td>
<td>2.9 (1.6, 3.5)</td>
<td>0.06</td>
</tr>
<tr>
<td>Tiff (pg/ml)</td>
<td>0.79 (0.34)</td>
<td>0.73 (0.40)</td>
<td>0.72 (0.40)</td>
<td>0.6</td>
</tr>
<tr>
<td>sTNF-R1 (ng/ml)</td>
<td>1.11 (0.16)</td>
<td>1.16 (0.17)</td>
<td>1.10 (0.17)</td>
<td>0.019</td>
</tr>
<tr>
<td>IL10 (pg/ml)</td>
<td>1.18 (1.41)</td>
<td>0.89 (1.18)</td>
<td>1.10 (1.19)</td>
<td>0.029</td>
</tr>
<tr>
<td>IL1&lt;sub&gt;1&lt;/sub&gt; (pg/ml)</td>
<td>0.12 (0.09, 0.23)</td>
<td>0.11 (0.08, 0.18)</td>
<td>0.11 (0.08, 0.14)</td>
<td>0.1</td>
</tr>
<tr>
<td>IL1Ra (pg/ml)</td>
<td>90 (54, 196)</td>
<td>54 (35, 85)</td>
<td>101 (59, 138)</td>
<td>0.001</td>
</tr>
<tr>
<td>IL6 (pg/ml)</td>
<td>0.16 (0.07)</td>
<td>0.15 (0.07)</td>
<td>0.16 (0.10)</td>
<td>0.8</td>
</tr>
<tr>
<td>MCP1 (pg/ml)</td>
<td>203 (165)</td>
<td>201 (150)</td>
<td>307 (230)</td>
<td>0.052</td>
</tr>
<tr>
<td>CCL21 (pg/ml)</td>
<td>244 (157)</td>
<td>236 (154)</td>
<td>245 (159)</td>
<td>0.08</td>
</tr>
<tr>
<td>OPG (ng/ml)</td>
<td>1.47 (0.51)</td>
<td>1.38 (0.47)</td>
<td>1.39 (0.40)</td>
<td>0.5</td>
</tr>
<tr>
<td>Neopterin (nM)</td>
<td>7.99 (1.90)</td>
<td>8.21 (1.90)</td>
<td>8.31 (1.76)</td>
<td>0.8</td>
</tr>
<tr>
<td>sCD40L (ng/ml)</td>
<td>2.41 (2.15)</td>
<td>7.16 (4.01)</td>
<td>8.33 (4.22)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Wilcoxon signed rank test comparing rosiglitazone versus placebo.

AU, arbitrary units.

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Serum and plasma levels of inflammatory parameters

Total leukocyte counts were reduced after rosiglitazone compared to placebo and primarily reflected a decrease in the numbers of neutrophils (Table 2). Also, CRP concentration decreased after rosiglitazone versus placebo, although the difference did not reach statistical significance \((P = 0.06)\) (Table 3). Uric acid is thought, at least partly, to reflect the degree of inflammation and oxidative stress (17), and notably, uric acid levels were significantly lower after rosiglitazone than placebo (Table 2). Chemokines like MCP1 are reported to play a major role in atherogenesis (reviewed by (18)), and MCP1 levels were decreased after rosiglitazone treatment compared to placebo, although the difference did not reach statistical significance \((P = 0.05)\). In contrast to these anti-inflammatory effects, the decrease in IL10 after rosiglitazone as compared with placebo may potentially reflect inflammatory effects of this medication (Table 3). IL1Ra is the natural inhibitor of IL1, possessing potent anti-inflammatory effects (19, 20), and notably, IL1Ra was significantly decreased on rosiglitazone treatment compared to placebo accompanied by no changes in IL1\(\beta\), suggesting inflammatory effects on the IL1 system (Table 3). As for the other markers, there were no significant effects of rosiglitazone as compared with placebo (Table 3).

Safety and tolerability

Three subjects developed mild to moderate pitting edema, and two subjects reported leg cramps while taking rosiglitazone. Liver transaminase concentrations were lower after rosiglitazone than placebo while creatinine concentrations were lower after placebo, but in general, the levels were within normal limit in both treatment groups (Table 2). There was a significant decrease in hemoglobin levels and red blood cell counts in the rosiglitazone group as compared with the placebo group, but again, the levels remained within normal limits (Table 2).

Effect of rosiglitazone on the IL1 system in vitro

A major finding in the present study was the significant decrease in plasma levels of IL1Ra after rosiglitazone treatment as compared with placebo treatment. To further elucidate this issue, we examined the ability of rosiglitazone to modulate the IL1 system in vitro. As can be seen in Fig. 1, in THP-1 monocytes that had been pre-activated with TNF-\(\alpha\) (5 ng/ml) before being exposed to different concentrations of rosiglitazone (Rosi) for 6 and 24 h. The cytokines were measured by ELISA, and data are presented as mean \(\pm\) S.E.M., \(n = 8\). \(*P < 0.05\) and \(**P < 0.001\) versus controls.

Discussion

PPAR\(\gamma\) agonists are widely used in the treatment of type 2 diabetes for their insulin-sensitizing properties (7). Also, there is evidence that TZDs exert a number of pleiotropic effects that may play an important role in the treatment of type 2 diabetes and potentially also in other cardiovascular disorders (12, 21). These effects include favorable outcome on blood pressure and potentially anti-inflammatory net effects. Most in vivo studies have been performed in patients with type 2 diabetes, but previously, rosiglitazone was shown to increase plaque collagen content and downregulate circulating levels of CRP and serum amyloid A in nondiabetic patients with symptomatic carotid artery stenosis (22). Moreover, previous reports have shown attenuating effects of rosiglitazone on plasma levels of IL6, IL18, and CRP in patients with MetSyn (3, 23). However, studies on the in vivo effects of rosiglitazone on inflammation have been somewhat conflicting, at least partly due to differences in study populations (e.g. healthy controls, obese patients, and patients with type 2 diabetes). In the present study, we extend previous findings by showing a marked downregulatory effect on plasma levels IL1Ra as the...
major finding. Based on the ability of IL1Ra to inhibit the binding of IL1 to its membrane-bound receptor (24, 25), these findings may suggest inflammatory net effects on the IL1 system during rosiglitazone therapy.

Although IL1Ra might be viewed as an acute phase protein, since it has been shown that the injection of IL1 into IL6, both being potent inducers of CRP, in humans leads to a rapid rise in blood IL1Ra levels (24), the decline in IL1Ra levels, the natural inhibitor of IL1α and IL1β (24–26), during rosiglitazone therapy suggests inflammatory net effects on the IL1 system. In fact, the decline in IL1Ra levels was accompanied by no changes in IL1β levels, further supporting inflammatory net effects on the IL1 system during such therapy (20). The IL1 system is upstream in the inflammatory cascade, and IL1-related pathways appear to be of major importance in various inflammatory conditions such as atherosclerosis (27) and several autoimmune disorders (28). IL1 has also been linked to obesity and MetSyn (29, 30), potentially contributing to the high risk of cardiovascular disease in these individuals. Adipose tissue is an important source of IL1Ra (31, 32). However, in the current study, rosiglitazone had no effect on body weight, BMI, or waist circumference suggesting more direct effects of rosiglitazone on the IL1 system. Nonetheless, the downregulatory effect of rosiglitazone on IL1Ra could represent a harmful effect of TZDs in MetSyn, potentially contributing to the recently reported increased risk of myocardial infarction (33) and chronic heart failure (34, 35) during such therapy.

In contrast to our in vivo data, our in vitro data in THP-1 monocytes suggest that rosiglitazone also may possess anti-inflammatory effects on the IL1 system, at least in some concentrations, with suppressive effects on IL1β levels combined with enhancing effects on IL1Ra. Similar anti-inflammatory effects on the IL1 system in vitro have also been reported by others in THP-1 monocytes (36) and synovial fibroblasts (37). Thus, although our in vivo data clearly suggest inflammatory net effect on the IL1 system during rosiglitazone therapy, representing the main finding in the present study, our in vitro data illustrate the complex effects of these medications, potentially exhibiting anti-inflammatory effects in certain tissues or cells at least at certain concentrations.

In addition to its inflammatory effect on the IL1 system, rosiglitazone was found to decrease IL10 levels further suggesting inflammatory effects of this medication. On the other hand, rosiglitazone increased plasma levels of sTNF-R1 and tended to decrease MCP1 and CRP, which may reflect anti-inflammatory effects. Furthermore, rosiglitazone markedly reduced uric acid levels. A raised level of uric acid is a phenotypic characteristic of patients with MetSyn, potentially reflecting enhanced inflammation and oxidative stress (17). The decrease in uric acid levels during rosiglitazone therapy could therefore mirror beneficial net effects on these processes in MetSyn. These findings suggest that the in vivo effects of TZDs may be rather complex including inflammatory but also potential anti-inflammatory effects.

During rosiglitazone therapy, there was an increase in serum levels of total cholesterol, reflecting an increase in LDL cholesterol without any significant changes in HDL cholesterol, and the apolipoprotein B/A1 ratio was increased. A similar pattern on lipid parameters has also been reported by others (reviewed by (38, 39)). It has been suggested that these side effects and their potential for cardiac risk must be weighed against the beneficial effects on glucose metabolism. In fact, recent studies have suggested that rosiglitazone may increase the risk of fractures and cardiac disease, although the cardiac disease observed was in the form of heart failure rather than ischemic heart disease events (33–35).

The present study has certain limitations such as a low number of subjects, and the study period was relatively short. Moreover, although the study was randomized and double-blind placebo-controlled, the cross-over design may have some limitations. Finally, caution is needed when interpreting data from multiple comparisons in a relatively small study population. Nevertheless, the current study shows potentially inflammatory effects of rosiglitazone in patients with MetSyn, primarily reflecting downregulatory effects on the naturally occurring IL1 inhibitor, IL1Ra. However, the effects of TZDs are rather complex, and their net effect on cardiovascular risk in MetSyn and other patient populations should be further investigated.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Rosiglitazone decreases IL1Ra


