Effect of PPAR-γ Agonist Treatment on Markers of Endothelial Dysfunction in Patients with Type 2 Diabetes Mellitus

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Received July 3, 2006
Accepted October 18, 2006
On-line available November 6, 2006

Summary
Thiazolidinediones are insulin-sensitizing drugs acting through peroxisome proliferator-activated receptor (PPAR)-γ. The aim of our study was to evaluate the effect of 5-month treatment with PPAR-γ agonist – rosiglitazone (4 mg/day), on the circulating markers of endothelial dysfunction and to evaluate the role of changes in endocrine function of adipose tissue in this process. Biochemical and metabolic parameters, circulating adiponectin, resistin, ICAM-1, VCAM-1, E-selectin, P-selectin, PAI-1, myeloperoxidase (MPO), and matrix metalloproteinase-9 (MMP-9) concentrations were assessed in 10 women with type 2 DM before and after rosiglitazone treatment and in a control group of healthy women. At baseline, diabetic group had significantly higher serum concentrations of glucose, glycated hemoglobin, V-CAM and PAI-1 compared to control group. Adiponectin levels tended to be lower in diabetic group, while resistin concentrations did not differ from control group. Rosiglitazone treatment improved diabetes compensation, significantly reduced VCAM-1, PAI-1 and E-selectin concentrations and increased adiponectin levels, while it did not affect serum resistin concentrations. Adiponectin concentrations at baseline were inversely related to E-selectin and MPO levels, this correlation disappeared after rosiglitazone treatment. We conclude that 5-month rosiglitazone treatment significantly reduced several markers of endothelial dysfunction. This effect could be at least in part attributable to marked increase of circulating adiponectin levels.

Key words
Diabetes mellitus • Endothelial dysfunction • Thiazolidinediones • Cardiovascular risk • Adipocyte-derived hormones

Introduction
Combination of visceral obesity, insulin resistance/diabetes, arterial hypertension, dyslipidemia and numerous other pathological states is now referred to as the metabolic or Reaven syndrome (Reaven 1988). Many epidemiological studies have demonstrated that presence of this syndrome markedly increases the risk of cardiovascular morbidity and mortality (Haffner 2003a,b, Haffner et al. 1998). Although the connection between the metabolic syndrome and cardiovascular complications is firmly established, the underlying pathophysiological...
mechanisms are only partially understood. One of the possible links between the metabolic syndrome and enhanced atherogenesis include a metabolic and endocrine dysfunction of adipose tissue with increased release of free fatty acids, enhanced production of insulin resistance-inducing and decreased production of insulin-sensitizing adipose tissue-derived hormones (Ravussin and Smith 2002, Haluzík et al. 2004, Haluzík and Haluzíková 2006). Therefore, one of the possible therapeutic approaches to prevent the development of atherosclerosis in patients with the metabolic syndrome is to improve metabolic and endocrine dysfunction of the adipose tissue.

One of the few drug classes with clearly established favorable effects on impaired endocrine function of the adipose tissue are thiazolidinediones (TZDs) (Olefsky 2000, Combs et al. 2002). These substances exert their effects by stimulating of peroxisome proliferators-activated receptors (PPAR)-γ that are most abundantly expressed in adipose tissue (Vamecq and Latruffe 1999). Peroxisome proliferator-activated receptors (PPARs) are nuclear transcription factors that regulate gene expression in response to activation by specific ligands (Fruchart et al. 2002, Haluzík and Haluzík 2006). PPARs are highly expressed in several tissues, including the adipose tissue, monocytes/macrophages and smooth muscle cells (Loviscach et al. 2000). Both experimental and clinical data clearly show that stimulation of PPAR-γ receptor by exogenous ligands such as thiazolidinediones improve insulin sensitivity, diabetes compensation and in some studies also blunt the atherogenic processes (Nolan et al. 1994, Saltiel and Olefsky 1996, Blaschke et al. 2006). Rosiglitazone – one of the powerful synthetic PPAR-γ agonists – is now widely used in the treatment of type 2 diabetes (Mayerson et al. 2002). Numerous studies also documented a positive effect of thiazolidinediones on circulating lipid levels namely decrease of triglycerides and fatty acids concentrations, and increase of HDL-cholesterol (Tan et al. 2005). Furthermore, TZDs exhibit many additional properties such as an important anti-inflammatory action and anti-atherogenic effects.

Here we tested the effect of chronic rosiglitazone treatment on selected metabolic and hormonal parameters in patients with type 2 diabetes mellitus. We hypothesized that activation of PPAR-γ receptors not only improves insulin sensitivity but also exerts anti-atherogenic effects in patients with type 2 diabetes. Furthermore, we tested the hypothesis that this effect may in addition improve diabetes compensation mediated by changes of endocrine function of adipose tissue. To this end, we measured circulating markers of endothelial dysfunction and atherosclerosis in patients with type 2 diabetes before and after treatment with PPAR-γ agonist rosiglitazone and correlated these parameters with circulating levels of adipose tissue-derived hormones adiponectin and resistin.

Patients and Methods

Study subjects

Ten female subjects with type 2 diabetes mellitus (DM duration 6.3±1.8 years) and ten sex-matched healthy controls were included in the study. The group of patients with type 2 DM had been treated with peroral antidiabetic drugs (metformine) in monotherapy for at least 6 months before the beginning of the study. Four patients with type 2 DM were treated with statins and another four of them with fibrates for at least 6 months before beginning of the study. Body weight of the subjects remained stable for at least three months before enrolment in the study. None of the studied subjects suffered from any acute infectious disease. Written informed consent was provided by all participants before being enrolled in the study. The study was approved by the Human Ethical Review Committee, First Faculty of Medicine and General University Hospital, Prague, Czech Republic.

Study protocol

Subjects were treated with rosiglitazone in a dose 4 mg/day for 5 months (148±5.3 days). All patients were examined in the basal state before the start of rosiglitazone administration and after 5 months of rosiglitazone treatment. Control subjects underwent only one physical examination and blood withdrawal and received no medication. All subjects were measured and weighed. Blood samples were withdrawn between 7:00 and 8:00 h after overnight fasting.

Hormonal and biochemical assays

Serum concentrations of soluble E-selectin (sE-Selectin), soluble VCAM-1 (sVCAM-1), soluble ICAM-1 (sICAM-1), matrix metalloproteinase-9 (MMP-9), myeloperoxidase (MPO), and total plasminogen activator inhibitor-1 (PAI-1) were measured by multiplex assay LINCOplex Kits (Linco Research, St. Charles, Missouri, USA). Sensitivity for analytes were sE-Selectin 79 pg/ml,
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sVCAM-1 16 pg/ml, sICAM-1 9 pg/ml, MMP-9 1 pg/ml, MPO 7 pg/ml, tPAI-1 11 pg/ml. The intra- and interassay variability were 4.5-12.3 % and 8.5-16.3 %, respectively. Serum adiponectin concentrations were measured by commercial ELISA kit (Linco Research, USA). Sensitivity was 1.0 ng/ml, and the intra- and interassay variability were 1.78 % and 9.25 %, respectively. Serum resistin concentrations were measured by commercial ELISA kit (BioVendor, Czech Republic). Sensitivity was 0.2 ng/ml and the intra- and interassay variability were 3.1 % and 6.5 %, respectively. Plasma glucose and lipids levels were measured by standard laboratory methods in the Department of Biochemistry of the General University Hospital.

Statistical analysis

The statistical analysis was performed on SigmaStat software (Jandel Scientific, USA). The results are expressed as means ± S.E.M. Data of diabetic patients before and after rosiglitazone treatment were compared by a paired t-test. Data of control subjects and of diabetic patients before and after rosiglitazone treatment respectively were compared by one-way analysis of variance followed by Dunn’s test. Correlations between adipocyte-derived hormones and endothelial dysfunction markers levels were evaluated by Spearman’s correlation test.

Results

Anthropometric values and parameters of diabetes compensation

At baseline, BMI in the DM2 group before treatment was significantly higher than in the control group. Rosiglitazone treatment did not significantly affect this variable (Table 1). Serum glucose and HbA1c concentrations in DM2 group were significantly higher than in the control group. Treatment with rosiglitazone significantly decreased glucose concentrations and tended to lower HbA1c concentrations (Table 1). Treatment with rosiglitazone significantly decreased glucose concentrations and tended to lower HbA1c concentrations (Table 1). Neither systolic or diastolic blood pressure values were significantly affected by rosiglitazone treatment (data not shown).

Adipose tissue-derived hormones and parameters of endothelial dysfunction

Baseline adiponectin concentration tended to be reduced in the diabetic group (15.5±1.9 vs. 25.2±7, p=0.08) while serum resistin concentrations were comparable between the groups. Rosiglitazone treatment significantly increased serum adiponectin levels (Fig. 1) but it did not affect serum resistin concentrations (Fig. 2).

At baseline, diabetic patients had higher total sVCAM-1 and PAI-1 concentrations relative to the control group (Table 2). There was a similar non-significant tendency for MMP-9, ICAM and E-selectin. MPO levels in the diabetic group were comparable to

Table 1. Anthropometric and biochemical parameters of control group of healthy women and subjects with type 2 DM before (DM group 1) and after (DM group 2) five months of rosiglitazone administration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n=10)</th>
<th>DM group 1 (n=10)</th>
<th>DM group 2 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54.9±1.3</td>
<td>63.3±2.7*</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1±1.1</td>
<td>29.9±1.2*</td>
<td>29.8±1.2*</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>1.6±0.6</td>
<td>3.7±1.2</td>
<td>2.5±0.6</td>
</tr>
<tr>
<td>gly (mmol/l)</td>
<td>4.7±0.3</td>
<td>8.9±0.6**</td>
<td>7.2±0.4**</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.0±0.1</td>
<td>5.4±0.2**</td>
<td>5.2±0.3*</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>1.1±0.1</td>
<td>1.4±0.1</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>Chol. (mmol/l)</td>
<td>5.6±0.2</td>
<td>4.1±0.7**</td>
<td>4.4±0.2**</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.7±0.1</td>
<td>1.2±0.1*</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.4±0.2</td>
<td>2.2±0.2*</td>
<td>2.3±0.4**</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. Statistical significance is from one-way ANOVA and Paired t-test respectively. * p<0.05 vs. control group, ** p<0.001 vs. control group; ø p<0.05 DM group 1 (before treatment) vs. DM group 2 (after treatment). BMI – body mass index, HbA1c – glycated hemoglobin.

Fig. 1. Adiponectin concentrations in control group of healthy women and subjects with type 2 DM before (DM group 1) and after (DM group 2) five months of administration of rosiglitazone. Values are means ± S.E.M. Statistical significance is from one-way ANOVA and paired t-test, respectively. ø p<0.05 DM group 1 (before treatment) vs. DM group 2 (after treatment).
those of the control group (Table 2). Treatment with rosiglitazone significantly decreased sVCAM, PAI-1 and E-selectin concentrations, while it did not significantly affect serum concentrations of sICAM, MPO and MMP-9 (Table 2).

### Relationship of circulating adiponectin and resistin levels to parameters of endothelial dysfunction

Adiponectin concentrations in diabetic group before rosiglitazone treatment negatively correlated with MPO ($r = -0.648$, $p<0.05$) and E-selectin ($r = -0.709$, $p<0.05$) levels. Baseline resistin levels tended to correlate positively with MPO levels ($r = 0.612$, $p=0.051$). No significant correlations were found between adiponectin and resistin and other endothelial dysfunction markers levels before and after treatment.

### Discussion

Obesity clusters with numerous abnormalities such as dyslipidemia, arterial hypertension or insulin resistance/diabetes and this combination is now commonly referred to as a metabolic or Reaven syndrome (Reaven 1988, 1992, 2001). The importance of this syndrome lies in the fact that its presence markedly increases the risk of atherosclerosis with subsequently enhanced cardiovascular morbidity and mortality (Haffner et al. 1996, Howard et al. 1998). Overt atherosclerotic changes of the vessel wall are usually preceded with its functional abnormalities such as increased rigidity, blunted response to vasodilatory agents and increased activation of endothelium (Esper et al. 2006). Increased endothelial activation is accompanied by enhanced production of endothelial molecules such as E-selectin, ICAM-1 and VCAM-1 that interact with other factors to promote atherogenesis (Huo and Ley 2001).

Due to the complex nature of interactions between components of the metabolic syndrome and atherosclerosis it is important to search for pharmacological approaches that can target several if not all components of metabolic syndrome at the same time. PPAR-γ agonists – thiazolidinediones represent one of the most promising candidates to fulfill the above mentioned requirements. They were originally discovered as insulin-sensitizing agents and used to treat diabetes and insulin resistance (Lehmann et al. 1995, Saltiel and Olefsky 1996). However, further studies have indicated that these drugs have a number of other beneficial effects beyond its insulin-sensitizing and blood glucose-lowering properties (Komers and Vrána 1998, Su et al. 1999, Moore et al. 2001). Here we have demonstrated that 5 months of treatment with potent PPAR-γ agonist rosiglitazone not only improved diabetes compensation but also decreased circulating levels of VCAM, PAI-1 and E-selectin, thus indicating improvement of endothelial dysfunction. It also tended to decrease circulating levels of matrix metalloproteinase-9 which has a proteolytic activity on connective tissue proteins and is involved in atherogenesis, vascular remodeling, and the creation and rupture of atherosclerotic plaques (Dandona et al. 2003). Although the baseline differences in circulating endothelial molecules between diabetic and control group could have been slightly affected by lower age of the latter group, our data (albeit on a relatively small number of patients)

### Table 2. Circulating markers of endothelial dysfunction in control subjects and subjects with type 2 DM before (DM group 1) and after (DM group 2) five months of rosiglitazone administration.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=10)</th>
<th>DM group 1 (n=10)</th>
<th>DM group 2 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9</td>
<td>133 ± 19.4</td>
<td>193 ± 24.8</td>
<td>130 ± 37.7</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>1287 ± 61</td>
<td>1501 ± 73.2*</td>
<td>1244 ± 110.1°</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>164.9 ± 35.5</td>
<td>195.8 ± 23.2</td>
<td>176.2 ± 17.8</td>
</tr>
<tr>
<td>PAI-1</td>
<td>28.6 ± 2.1</td>
<td>46.4 ± 5.1°</td>
<td>36.6 ± 3.9°</td>
</tr>
<tr>
<td>MPO</td>
<td>58.3 ± 13.3</td>
<td>62.1 ± 19.3</td>
<td>61.29 ± 20.1</td>
</tr>
<tr>
<td>E-sel.</td>
<td>18.2 ± 2.4</td>
<td>26.2 ± 4.4</td>
<td>21.0 ± 3.3°</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. Statistical significance is from one-way ANOVA and paired t-test, respectively. * $p<0.05$ vs. control group, ° $p<0.05$ DM group 1 (before treatment) vs. DM group 2 (after treatment). ICAM-1 – intercellular adhesion molecule 1, VCAM-1 – vascular cell adhesion molecule 1, MPO – myeloperoxidase, MMP-9 – matrix metalloproteinase-9.
show a clear improvement of endothelial dysfunction markers after rosiglitazone treatment in patients with type 2 diabetes. This finding is in agreement with previously published studies showing that thiazolidinediones improve the mechanic properties of the vessel wall (Caballero et al. 2003, Esposito et al. 2006), although it has to be noted that not all of the clinical studies supported this concept (Buras et al. 2005).

There are several possible explanations of the beneficial effects of rosiglitazone treatment on endothelial dysfunction in our study. Firstly, chronically increased blood glucose levels as seen in patients with diabetes have numerous negative metabolic effects including a direct proatherogenic action through a multiple mechanism (Ceriello 2005, Lim et al. 2006). Therefore the improvement of diabetes compensation could have affected endothelial activation by itself. Another unifying hypothesis between obesity, insulin resistance and atherosclerosis concerns impaired endocrine function of adipose tissue with increased production of proinflammatory and insulin resistance-inducing factors and decreased production of insulin-sensitizing hormones (Havel 2002). Here we have focused on the possible involvement of two adipose tissue-derived hormones that have been implicated in the etiopathogenesis of both insulin resistance and atherosclerosis. First one, adiponectin, is a protein hormone produced almost exclusively by adipocytes with important insulin-sensitizing properties (Berg et al. 2002). Furthermore, transgenic adiponectin-knockout mice also exhibit exaggerated atherosclerosis that is blunted by adiponectin replacement (Kubota et al. 2002). Clinical studies have shown that decreased adiponectin levels are linked to accelerated atherosclerosis (Shimada et al. 2004). Another adipose tissue-derived hormone studied herein, resistin, possesses properties that are in many respects opposite to those of adiponectin. It has been reported to be higher in rodent models of obesity and insulin resistance and it has been shown to promote atherogenesis (Steppan et al. 2001, Kawanami et al. 2004). Clinical studies have not fully supported its role as a link between obesity and insulin resistance but most of them linked increased resistin levels to increased risk of atherosclerosis (Kawanami et al. 2004, Anderlová et al. 2005, Pischon et al. 2005). Here we have shown in accordance with previously published studies that rosiglitazone administration markedly increased circulating adiponectin levels which in turn may have directly affected endothelial activation and possibly also circulating MMP-9 levels. It has been demonstrated previously that adiponectin levels are lower in patients with microalbuminuria relative to those without microalbuminuria indicating a relationship of this hormone to microalbuminuria as another measure of endothelial dysfunction (Tsioufis et al. 2005). Our data indicate that activation of PPAR-γ receptors can target atherosclerosis at least in part through the stimulation of adiponectin production in adipose tissue. Furthermore, since PPAR-γ receptors are also present in circulating monocytes and macrophages, it is possible that rosiglitazone can also directly affect this potential source of proatherogenic and proinflammatory factors.

In contrast to adiponectin levels clearly influenced by rosiglitazone treatment we did not observe any effect of PPAR-γ activation on circulating resistin levels. This observation underlines relatively controversial findings regarding the importance of resistin in the etiopathogenesis of insulin resistance and atherosclerosis in humans. Firstly, resistin in humans appears (in contrast to rodents) to be produced predominantly, if not exclusively, by immunocompetent cells rather than by adipocytes (Savage et al. 2001). Secondly, while some studies showed increased circulating resistin levels in human obesity and type 2 diabetes, many others including our data failed to do so (McTernan et al. 2002, Anderlová et al. 2005). Finally, it has been shown that resistin secretion is strongly activated by endotoxin administration indicating that its major role in humans may lie rather in the regulation of inflammation than insulin sensitivity (Lehrke et al. 2004). Here we did not see any effect of rosiglitazone treatment on the circulating resistin levels despite a clear improvement of diabetes compensation and endothelial dysfunction, although we cannot exclude the possibility that resistin production was affected only locally in adipose tissue without major changes in systemic resistin concentrations. Taken together, our study does not support the role for resistin as a link between obesity, insulin resistance and atherosclerosis in humans.

In conclusion, we have demonstrated that treatment with PPAR-γ agonist rosiglitazone for 5 months markedly improved diabetes compensation and circulating markers of endothelial dysfunction. The beneficial effect on endothelial dysfunction can very likely be explained by the combination of glucose-lowering effect of rosiglitazone and by increased circulating levels of anti-atherogenic hormone adiponectin. The direct influence of PPAR-γ activation
combined with increased adiponectin levels probably affected the production of endothelial dysfunction markers in both the vasculature and adipose tissue that has been previously shown as a source of these molecules especially in subjects with obesity (Plomgaard et al. 2005). In contrast, resistin levels were not affected by rosiglitazone treatment in our study suggesting that this hormone in not involved in the improvement of insulin sensitivity and endothelial dysfunction after rosiglitazone treatment.

Acknowledgements
Supported by Research Project of MH CR No. 64165.

References


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