Short-term treatment with rosiglitazone improves glucose tolerance, insulin sensitivity and endothelial function in renal transplant recipients

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Abstract

Background. Insulin resistance (IR) contributes to the development of glucose intolerance (post-transplant diabetes mellitus or impaired glucose tolerance) following renal transplantation. Furthermore, endothelial dysfunction (ED) is associated with IR. Glucose intolerance, IR and ED are all independent risk factors for cardiovascular disease. Therefore, treatment with insulin sensitizers may benefit glucose-intolerant renal transplant recipients. The main objectives of the present study were to investigate the effect of 4 weeks’ treatment with the PPAR-γ agonist rosiglitazone on insulin sensitivity, plasma glucose and endothelial function in renal transplant recipients with glucose intolerance. Safety parameters were also addressed.

Methods. A total of 10 glucose-intolerant renal transplant recipients were treated with rosiglitazone (initially 4 mg/day increasing to 8 mg/day after 1 week). A hyperinsulinaemic euglycaemic glucose clamp, an oral glucose tolerance test and endothelial function assessment with laser Doppler flowmetry were performed both at baseline and at follow-up.

Results. Treatment with rosiglitazone was followed by a significantly improved mean glucose disposal rate (from 6.5 to 9.1 g/kg/min; \( P = 0.02 \)) and a significant decline in fasting and 2 h plasma glucose (from 6.4 to 5.8 mmol/l, \( P = 0.01 \) and from 14.2 to 10.6 mmol/l, \( P = 0.03 \), respectively). Furthermore, a significant improvement in endothelial function was demonstrated (\( \text{AUC}_{\text{ACH}} \); from 389 to 832 AU min, \( P = 0.04 \)). No serious adverse events or hypoglycaemic episodes were observed.

Conclusions. Four weeks’ treatment with rosiglitazone was associated with increased insulin sensitivity, lowered fasting and 2 h plasma glucose and improved endothelial function in renal transplant recipients with glucose intolerance. The drug was well tolerated and may be a good alternative for treating these patients.

Keywords: endothelium; glucose intolerance; insulin resistance; renal transplantation; thiazolidinediones

Introduction

Post-transplant diabetes mellitus (PTDM) is associated with reduced graft and patient survival in renal transplant recipients [1]. Moreover, PTDM and impaired glucose tolerance (IGT) is associated with a clustering of traditional cardiovascular risk factors and metabolic abnormalities consistent with a post-transplant metabolic syndrome [2].

Insulin resistance (IR) has a pivotal role in the development of PTDM [3] and increased daily prednisolone dose is an independent risk factor for IR following renal transplantation [4].

Endothelial dysfunction (ED), defined as impaired endothelium-dependent vasodilatation, is also a prevalent feature among renal transplant patients [5]. ED involves an imbalance between vasoconstrictive and vasodilative substances and may predict future cardiovascular events. IR and ED have been reported to coexist in many clinical conditions, such as the metabolic syndrome, obesity and type 2 diabetes [6]. Treatment with rosiglitazone, a peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist, has been associated with a significantly improved endothelial function in non-diabetic patients with the metabolic syndrome [7].

Binding of rosiglitazone to its nuclear receptor leads to altered transcription of genes involved in the regulation of lipid and glucose metabolism. Interestingly, the prescription of the PPAR-γ agonist troglitazone to healthy individuals treated with dexamethasone led to a complete reversal of the glucose
intolerance and IR [8]. Such an effect may also be relevant for solid-organ transplant recipients receiving glucocorticoids.

Conventional oral antidiabetic drugs, such as long-acting sulphonylureas and metformin, may cause serious side effects (hypoglycaemia and lactacidosis, respectively) in patients with renal dysfunction. Taking this into account, the traditional oral hypoglycaemic agents may not be optimal for treating renal transplant recipients. The new insulin sensitizer rosiglitazone may represent an attractive alternative for several reasons. The drug is safe even in patients with renal failure [7] and is not metabolized by the CYP3A4 system, thus, lowering the risk for interactions with calcineurin inhibitors. In addition, rosiglitazone may have specific advantages because it is reported to counteract the effects of steroids on glucose homeostasis and may even improve endothelial function [7,8].

The main objectives of the present study were to investigate the impact of 4 weeks' treatment with the PPAR-γ agonist rosiglitazone on insulin sensitivity, plasma glucose and endothelial function in renal transplant recipients with glucose intolerance. In addition, safety parameters were addressed.

Subjects and methods

Patients

A total of 13 Caucasian renal transplant recipients (12 males/one female) were recruited to this open study. None of the participants had diabetes prior to transplantation. As part of the inclusion criteria for this study, the patients underwent an oral glucose tolerance test (OGTT) to determine the glucose tolerance. Based on the OGTT, seven recipients had PTDM and three had IGT. Three recipients (all males) were classified as normoglycaemic and were not treated with insulin: 8 mg sid and this dose was given throughout the study period. One reason for a relatively short treatment period was to avoid the confounding effect of prednisolone tapering [4]. All patients completed a hyperinsulinaemic euglycaemic glucose clamp (HEC), an OGTT and endothelial function assessment with laser Doppler flowmetry (LD) at baseline and at follow-up. Blood samples for analysing total plasma cholesterol, LDL- and HDL-cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, free fatty acids (FFA), endothelin-1 (ET-1) and nitric oxide were drawn both at baseline and following 4 weeks' treatment. Rosiglitazone was given in blister packages and compliance was controlled by tablet count at days 7 and 28.

Hyperinsulinaemic euglycaemic glucose clamp

A HEC was performed in order to assess the insulin sensitivity as described previously [9]. Insulin was infused at a fixed rate of 1 mU/kg/min. Blood glucose was clamped at 5 mmol/l. This was obtained by measuring glucose concentrations every 5 min and adjusting the rate of intravenous glucose infusion (concentration: 250 mg/l). A blood glucose of 5 mmol/l was maintained for 120 min and the glucose disposal rate [GDR (mg/kg lean body mass/min)] was calculated from the amount of glucose infused during steady state in the last 60 min [10]:

\[
\text{GDR} = \frac{\text{Mean glucose infusion rate last 60 min (ml/h)} \times \text{Glucose concentration (mg/ml)}}{\text{Lean body mass (kg)}}
\]

Steady state levels of insulin varied from person to person. Therefore, the insulin sensitivity index (ISI) was calculated. ISI is a measure of the tissue sensitivity for the attained insulin, i.e. the amount glucose metabolized per unit of serum insulin:

\[
\text{ISI} = \frac{\text{GDR}}{\text{Mean insulin concentration last 60 min}}
\]

Oral glucose tolerance test

The participants were given a 75 g oral glucose load. Venous blood samples were drawn immediately before and at 30, 60, 90 and 120 min. Whole blood glucose was analysed using HemocueAB® B-glucose Analyser (Ängelholm, Sweden) and calculated to plasma values [11]. The recipients were
diagnosed according to the criteria suggested by the Expert Committee [12]: PTDM with fasting plasma glucose ≥7.0 mmol/l or 2 h plasma glucose ≥11.1 mmol/l; IGT with fasting plasma glucose <7.0 mmol/l and 2 h glucose 7.8–11.0 mmol/l; impaired fasting glucose with fasting plasma glucose 5.6–6.9 mmol/l; and normal glucose tolerance with fasting plasma glucose <5.6 mmol/l and 2 h plasma glucose <7.8 mmol/l.

Serum insulin was analysed using a fluoroimmunoassay (Auto DELFIA™ Insulin; Wallac Oy, Turku, Finland). The total insulin response was estimated using the trapezoidal rule, calculating the area under the insulin vs time curve (AUCins) and the area under the glucose vs time curve (AUCgluc):

\[
\text{SEC}_{\text{AUC}} = \frac{\text{AUC}_{\text{ins}}}{\text{AUC}_{\text{gluc}}}
\]

The first- and second-phase insulin releases were estimated according to the following equations:

\[
\text{Secr}_1 = 1194 + 4.724 \times \text{Ins}_0 - 117.0 \times \text{Gluc}_1 + 1.414 \times \text{Ins}_1
\]

\[
\text{Secr}_2 = 295 + 0.349 \times \text{Ins}_1 - 25.72 \times \text{Gluc}_1 + 1.107 \times \text{Ins}_0
\]

where \(\text{Ins}_0\) and \(\text{Ins}_1\) are serum insulin levels at 0 and 1 h after an OGTT, respectively, and \(\text{Gluc}_1\) represents serum glucose 1 h post-load [13].

**Microvascular function investigation**

The endothelial function was estimated by stimulating the endothelium in the arterioles in the forearm by transdermal iontophoresis of acetylcholine (AUCACH) and by performing post-occlusive reactive hyperaemia test (AUCrh). The change in blood flow was measured by LD and the effect parameter used was the area under the flux vs time curve (AUC) following each stimulation (AUCACH and AUCrh, respectively). The method has been described in detail elsewhere [14].

**Safety**

As part of the safety routine, any changes in trough concentrations of CsA and tacrolimus, as well as the creatinine concentrations, were measured. Also, possible changes in routine haematology parameters and plasma AST/ALT levels until follow-up were detected.

**Statistical analysis**

The results are given as means±SD if not otherwise explained. The PTDM group has been analysed separately for the parameters GDR, fasting and 2 h plasma glucose. The statistical analyses were performed by the use of SPSS 12.0 for Windows®. The Student’s  t-test was used for comparing paired data. P-values of <0.05 were considered statistically significant.

Based on clinical experience, a 20% increment in insulin sensitivity was considered clinically relevant. Taking into account that 10 recipients were included, the present study had a power of >80% (\(\alpha=0.05\)) to detect a 20% increase in insulin sensitivity, measured as GDR.

**Results**

**Insulin resistance**

The mean GDR increased by 40% (from 6.5±2.9 to 9.1±4.0 mg/kg/min; \(P=0.02\)) after treatment with rosiglitazone (Figure 1). The results were essentially the same for the PTDM group alone (6.2±2.1 mg/kg/min at baseline and 9.1±4.4 mg/kg/min at follow-up; \(P=0.07\)). Four out of 10 patients had a pronounced improvement in GDR. The mean ISI increased (from 17.7±11.8 to 20.0±9.2 \(\times 10^{-9}\) mg·l/kg·min·pmol), although it was not statistically significant (\(P=0.24\)), during the treatment period.

**Oral glucose tolerance test**

The mean fasting plasma glucose was substantially lowered following treatment (from 6.4±2.1 mmol/l before to 5.8±2.1 mmol/l after treatment; \(P=0.01\)). The mean 2h plasma glucose levels fell significantly from 14.2±4.2 to 10.6±4.4 mmol/l (\(P=0.03\)) (Figure 2). The results were essentially the same for the PTDM group analysed alone (fasting plasma glucose declined from 7.0±2.2 mmol/l at baseline to 6.3±2.2 mmol/l at follow-up, \(P=0.07\), and 2 h plasma glucose was reduced from 16.0±3.5 mmol/l at baseline to 11.2±5.1 mmol/l at follow-up, \(P=0.03\)). Furthermore, the mean total glucose concentration during OGTT (AUCgluc) was significantly reduced (from 25.1±6.0 to 20.5±6.3 mmol·h/l; \(P<0.01\)).

**Insulin secretion**

Neither the mean OGTT-derived SEC_{AUC} nor estimated first and second phase insulin secretion were changed significantly during the study period.

**Lipids**

The mean concentration of fasting FFA was not changed during the study. There was a significant
increase in LDL-cholesterol ($P = 0.04$). Total cholesterol, HDL-cholesterol and triglycerides, however, did not change significantly.

**BMI and lean body mass**

BMI increased by a mean of $0.6 \pm 0.8 \, \text{kg/m}^2$ ($P = 0.06$) and lean body mass increased by $0.4 \pm 0.7 \, \text{kg}$ ($P = 0.08$) during the study.

**Microvascular endothelial function**

Rosiglitazone treatment was associated with an increase in LDL-cholesterol ($P = 0.04$). Total cholesterol, HDL-cholesterol and triglycerides, however, did not change significantly.

Rosiglitazone treatment was associated with an increase in acetylcholine-stimulated vasodilatation (endothelial-dependent function). Due to technical problems with the laser Doppler flowmeter, the endothelial function could not be assessed in two recipients. A total of five out of eight patients had an increase in AUC$_{ACh}$ (Figure 3). On average, the AUC$_{ACh}$ increased from $389 \pm 304 \, \text{AU} \cdot \text{min}$ at baseline to $832 \pm 492 \, \text{AU} \cdot \text{min}$ at follow-up ($P = 0.04$) (Figure 3). No significant linear association between the lowering in plasma glucose and the improvement in the endothelial function was found. Overall microvascular function (AUC$_{rh}$), estimated from the reactive hyperaemia test, was not changed.

**Vasoactive substances**

Nitric oxide concentrations (plasma and urine) and plasma ET-1 levels were not significantly changed during treatment with rosiglitazone.

**Safety parameters**

Rosiglitazone treatment was well tolerated. No changes in whole blood concentrations of CsA or plasma concentrations of tacrolimus were observed. Liver enzymes, routine haematology parameters and creatinine concentrations remained unchanged during the study period. Three recipients reported tremor, abdominal pain or oedema with uncertain relationship to rosiglitazone treatment.

**Discussion**

The major findings of the present study are that rosiglitazone may have a positive impact on insulin sensitivity and endothelial function in glucose-intolerant renal transplant recipients. No serious adverse effects of rosiglitazone were registered short-term.

**Insulin sensitivity**

The results of the present study support previous findings in non-transplanted patients, showing significant improvement in GDR after rosiglitazone treatment [15,16]. Our finding of a 40% increase in mean GDR in patients receiving 5–15 mg prednisolone per day is in line with the report by Willi et al. [8]. They found that healthy individuals treated with both troglitazone (400 mg/day) and dexamethasone 4 mg/day (equipotent to 20 mg prednisolone) had an ~50% higher GDR than those treated with dexamethasone alone.

In our study, ISI, which accounts for both GDR and the prevailing insulin levels, increased only non-significantly. The variability in both components of the ISI probably makes this a type II error. Importantly, this study was powered to detect a clinically significant effect of rosiglitazone treatment on insulin sensitivity measured as GDR.

**Plasma glucose**

Both fasting and, in particular, 2 h plasma glucose concentrations were lowered at the end of the treatment.
period, in accordance with the findings in non-transplanted patients [15–17]. Interestingly, several studies included in a European meta-analysis reported that especially elevated 2h plasma glucose concentrations indicate a risk of all-cause and cardiovascular mortality in a general population with or without known diabetes mellitus [18].

Microvascular function

To our knowledge, we are the first to show that renal transplant recipients with glucose intolerance improve their endothelial function even after a short time of treatment with rosiglitazone. Several studies have shown that improvement in glucose metabolism, regardless of plasma glucose-lowering therapy, improves the endothelial function [19]. However, we were not able to detect any significant association between the lowering in plasma glucose and the improvement in acetylcholine-stimulated vasodilation. Moreover, in the study by Wang et al. [7], rosiglitazone treatment correlated with improved endothelial function even though fasting plasma glucose did not change significantly. The positive effect on endothelial function by rosiglitazone may, therefore, be independent of its glucose-lowering effect.

We were able to detect a specific improvement in endothelial function (acetylcholine-mediated effect), whereas the overall microvascular function, including both endothelial cell and smooth vascular cell function (reactive hyperaemia test), remained unchanged. One possible explanation for this selective endothelial improvement could be due to a specific effect of rosiglitazone on endothelial cells and it has been shown that PPAR-γ receptors are present on endothelial cells [20].

Safety

The drug was well tolerated and all 10 recipients completed the study. Furthermore, all of the participants showed complete compliance. Rosiglitazone is not metabolized by CYP3A4. Therefore, interactions with immunosuppressive drugs, like CsA or tacrolimus, were not expected and, accordingly, we found no changes in the trough concentrations of CsA or tacrolimus. This is in line with a recent report [21]. Moreover, no increment in plasma creatinine or AST/ALT was shown. The recipients tended to show an increased BMI during the study. This weight increment may be explained by increased adipocyte differentiation, increased appetite or water retention [22].

Limitations

This study has some limitations. First, it is an uncontrolled study where the recipients serve as their own controls. The best study design would probably be a crossover study including a comparator group receiving either placebo or an oral antidiabetic drug. Second, the relatively short duration of the treatment time limits any comparison with other studies with longer treatment periods. However, the short treatment period and the fact that the patients tended to gain weight would probably underestimate the potential glucose-lowering effect of rosiglitazone.

In conclusion, 4 weeks’ treatment with rosiglitazone was associated with increased insulin sensitivity, lowered fasting and 2 h plasma glucose and improved endothelial function in renal transplant recipients with glucose intolerance. The medication was well tolerated and rosiglitazone may be a good treatment alternative for this patient population.

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Conflict of interest statement. None declared.

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