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Impact of rosiglitazone and glyburide on nitrosative stress and myocardial blood flow regulation in type 2 diabetes mellitus

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Abstract

Cardiovascular disease, the leading cause of death in patients with type 2 diabetes mellitus (T2DM), is usually preceded by endothelial dysfunction and altered myocardial blood flow (MBF) regulation. Hyperglycemia, oxidative-nitrosative stress, systemic inflammation, and insulin resistance are implicated in the pathogenesis of abnormal MBF regulation, myocardial ischemia, and apoptosis. However, the impact of oral antihyperglycemic therapy on myocardial perfusion is controversial. Our objective was to explore the effect of rosiglitazone and glyburide on nitrosative stress and MBF regulation in subjects with T2DM. [^{13}N]ammonia positron emission tomography and cold pressor testing were used in 27 diabetic subjects (mean age, 49 ± 11 years; glycohemoglobin, 7% ± 1.5%) randomized to either rosiglitazone 8 mg/d or glyburide 10 mg/d for 6 months. Isotope dilution gas chromatography–mass spectrometry was used to quantify plasma 3-nitrotyrosine, a stable marker of reactive nitrogen species. At 6 months, there were no significant differences between groups in the mean glycohemoglobin, blood pressure, or plasma lipids. Rosiglitazone significantly reduced plasma nitrotyrosine, high-sensitivity C-reactive protein, and von Willebrand antigen ($P < .03$ for all) and significantly increased plasma adiponectin ($P < .05$). No significant changes in these parameters were observed with glyburide. Treatment with glyburide, but not rosiglitazone, resulted in a significant deterioration in both resting and stress MBF. Rosiglitazone, but not glyburide, ameliorated markers of nitrosative stress and inflammation in subjects with T2DM without impairing myocardial perfusion.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in type 2 diabetes mellitus (T2DM) [1-3] and is typically preceded by endothelial dysfunction [4] and impaired myocardial blood flow (MBF) regulation [5-7]. Subjects with diabetes commonly exhibit abnormal coronary vascular responsiveness [5-7] that may exacerbate regional myocardial ischemia during stress and increase the mortality associated with myocardial infarction [8]. The etiology of alterations in vascular reactivity complicating diabetes is unclear, but increased insulin resistance has been implicated [2]. Insulin resistance has been found to correlate with multiple cardiovascular risk factors including impaired glucose tolerance, dyslipidemia, hypertension, decreased fibrinolytic activity, systemic inflammation, and increased oxidative stress [9]. In man, reduced intracellular antioxidant defense is associated with insulin resistance [10].

Oxidative stress and systemic inflammation are implicated in the pathogenesis of diabetic atherosclerosis [11]. Hyperglycemia can increase oxidative stress via several mechanisms including increased nonenzymatic glycation and glycoxidation, activation of vascular nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase and myeloperoxidase, and production of reactive nitrogen species (RNS) and by overproduction of superoxide by the mitochondrial electron transport chain [12]. Recently, increased production of RNS and formation of nitrotyrosine—a highly specific oxidative product of tyrosine by

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RNS—have been identified in human subjects with CVD, implicating nitrotyrosine as a novel biomarker of RNS in vivo [12,13].

In turn, oxidative stress can have multiple detrimental downstream consequences including the activation of poly (adenosine diphosphate–ribose) polymerase [14], mitogen-activated protein kinases, cyclooxygenase-2, inducible nitric oxide synthase, cell adhesion molecules, and various inflammatory mediators [15]. Attenuation of hyperglycemia, reduction of insulin resistance, and decreased oxidative stress could therefore be expected to improve myocardial perfusion and decrease cardiovascular risk. However, there is considerable controversy whether drugs commonly used to treat T2DM—that is, the insulin sensitizer rosiglitazone and sulfonylureas—adversely impact CVD risk. A recent meta-analysis implicated rosiglitazone as a causative factor in myocardial ischemia and adverse cardiovascular events within 6 months of initiation [16]. The effect of sulfonylurea therapy on cardiac risk and event rate also remains unclear [17].

In this study, we sought out to explore the short-term (6 months) effects of rosiglitazone and glyburide therapy on plasma nitrotyrosine levels and MBF regulation in T2DM subjects free from known CVD.

2. Subjects and methods

2.1. Subjects

Subjects with T2DM without known coronary artery disease (ie, no angina, ischemic changes or left ventricular hypertrophy on resting electrocardiogram, or evidence of MBF deficits on resting positron emission tomography [PET] imaging) were recruited from the Michigan Diabetes Research and Training Center at the University of Michigan and the Endocrinology Clinics at the University of Toledo Medical Center. Other inclusion criteria were as follows: established T2DM [18], glycohemoglobin (HbA_{1c}) between 6% and 9% at baseline, treatment with diet/exercise or sulfonylurea therapy alone (or, if on insulin, <20 U/d, which could be safely converted to sulfonylurea monotherapy). Patients previously on metformin were entered after a 4-week washout period. No changes in lipid-lowering therapy were permitted throughout the course of the study. The study protocol was approved by the institutional review boards of both institutions.

A total of 27 T2DM subjects matched for age and diabetes duration were randomized to either rosiglitazone 8 mg/d or glyburide 10 mg/d for 6 months. Baseline clinical characteristics of the study groups are shown in Table 1.

The mean age for the cohort was 49.5 ± 10 years (range, 26-60 years), mean diabetes duration was 7.3 ± 6.4 years, and mean HbA_{1c} was 6.9% ± 1.6%. There were 14 male and 13 female subjects. Fourteen subjects were randomized to rosiglitazone; and 13 subjects, to glyburide. Randomization was equal in both arms except for sex, with more women randomized to rosiglitazone (n = 10). There was an equal distribution for statin users between the 2 groups.

Table 1
Baseline and end-of-study characteristics of study subjects

Variable	Baseline		End of study	
	Rosiglitazone	Glyburide	Rosiglitazone	Glyburide
HbA_{1c} (%)	6.5 ± 1	7.5 ± 1.9	6.0 ± 0.7	6.4 ± 0.9
Total cholesterol (mg/dL)	190 ± 44	202 ± 24	179 ± 70	180 ± 31
HDLc (mg/dL)	44 ± 10	45 ± 14	48 ± 14	45 ± 16
LDLc (mg/dL)	115 ± 37	111 ± 24	102 ± 54	100 ± 29
Triglyceride (mg/dL)	166 ± 92	226 ± 124	147 ± 73	173 ± 123
Systolic BP (mm Hg)	130 ± 22	137 ± 23	135 ± 22	136 ± 18
Diastolic BP (mm Hg)	73 ± 10	78 ± 11	76 ± 12	75 ± 11

Data are expressed as mean ± 1 SD. BP indicates blood Pressure; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol.

2.2. PET studies

Cardiac PET imaging was performed with 1 of 3 available whole-body PET scanners (Siemens ECAT/931, Siemens/ECAT Exact, or Siemens/ECAT Exact HR+, New York, NY) as previously reported [19]. Two separate PET imaging sessions assessed MBF regulation at baseline and at study end. [^{13}N]ammonia studies were conducted under resting conditions and during cold pressor testing (CPT) sequentially in a single PET imaging session. The resting perfusion scan was started by initiating a 15-minute dynamic PET acquisition sequence as 20 mCi of [^{13}N]ammonia was intravenously injected, as previously described [20]. The dynamic acquisition sequence consisted of 20 image frames (frame rates: 12 × 10, 6 × 30, and 2 × 300 seconds). After 50 minutes, a cold pressor stress [^{13}N]ammonia study was performed with cold pressor stimulation starting 30 seconds before tracer injection. Blood pressure and heart rate were recorded at 1-minute intervals throughout the entire study. All PET scans for a given patient were performed on the same scanner. Regional MBF was estimated from the measured [^{13}N]ammonia kinetics [20]. Regional MBF reserve values were estimated by dividing the stress flow value by the resting flow value. Mean values of resting flow, stress flow, and MBF reserve were then determined.

2.3. Cold pressor testing

The cold face test to provoke sympathetic activation was performed by applying a bag filled with ice chips to the forehead for 2 minutes as previously described [19].

2.4. Plasma nitrotyrosine

Plasma nitrotyrosine was determined by isotope dilution gas chromatography–mass spectrometry as described previously [21]. Plasma proteins were precipitated with ice-cold trichloroacetic acid (10% vol/vol), collected by centrifuga-

tion, washed with 10% trichloroacetic acid, and delipidated twice with water/methanol/water-washed diethyl ether (1:3:7, vol/vol/vol). Isotopically labeled internal standards were added, and samples were hydrolyzed with 4 N methanesulfonic acid. All samples were manually injected using an on-column injector and a Hewlett-Packard 6890 gas chromatograph (Palo Alto, CA) equipped with a 15-m DB-5 capillary column (0.25-mm internal diameter, 0.33-μm film thickness; J&W Scientific, Folsom, CA) interfaced with a Hewlett-Packard 5973 mass detector. The *t*-butyl dimethylsilyl derivatives of amino acids were quantified by selected ion monitoring using isotope dilution negative-ion chemical ionization gas chromatography–mass spectrometry [8]. Results are normalized to protein content of Tyr, the precursor of 3-nitrotyrosine.

2.5. Laboratory measurements

Venous plasma and serum samples were obtained after overnight fast at 8:00 AM. High-sensitivity C-reactive protein (hsCRP) was measured using particle-enhanced immunonephelometry (Prostec nephelometer, Dade Behring, New York, NY). Von Willebrand factor (vWF) antigen was measured using an STA-Liatest vWF kit (Diagnostica STAGO, Parsippany, NJ) and a Behring Coagulation System. Adiponectin was measured by enzyme-linked immunosorbent assay (Chemicon, Temecula, CA). Plasma glucose was assessed by glucose oxidase method, HbA_{1c} level was assessed by high-performance liquid chromatography, and serum cholesterol and triglyceride concentrations were measured using standard enzymatic methods via automated analyzer.

2.6. Statistical methods

Statistical analysis was performed using Graphpad Prism version 3.00 (Graphpad Software, San Diego, CA). Data are presented as mean ± SD. Data were screened for outliers (extreme or improbable values) and invalid values before analysis. Distributions of continuous measures were assessed for symmetry, and transformations (log or square root) were applied as necessary. All analyses were then performed on the transformed data. A 2-sample *t* test was used to test the equality of means between the 2 groups. The significance of changes in measurements from baseline was determined using a paired *t* test. Differences were considered significant at *P* less than or equal to .05.

3. Results

3.1. Effects of intervention on glucose, blood pressure, and lipid parameters

No significant differences in HbA_{1c}; blood pressure (systolic/diastolic); serum total, high-density lipoprotein, or low-density lipoprotein cholesterol; and triglycerides levels were found between groups after 6 months (Table 1).

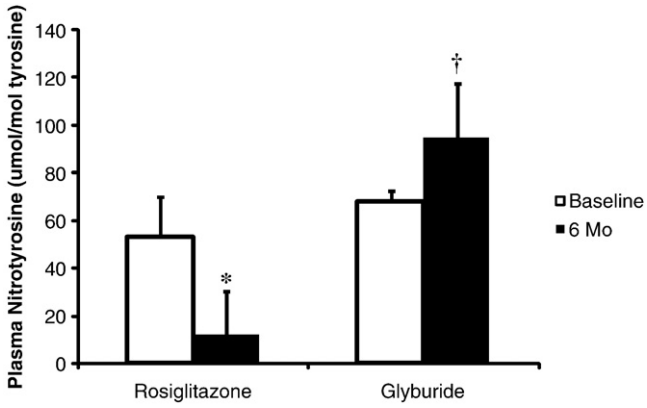

Fig. 1. Effects of rosiglitazone and glyburide on plasma nitrotyrosine. Plasma nitrotyrosine was measured at baseline and end of study as described in "Subjects and methods." Data are expressed as mean ± SEM. *P less than .05 vs baseline; †P less than .01 vs rosiglitazone.

3.2. Plasma nitrotyrosine

There were no significant differences in plasma 3-nitrotyrosine levels between rosiglitazone-and glyburide-treated subjects at baseline. However, plasma 3-nitrotyrosine levels in both groups of diabetic subjects were approximately 5-fold higher compared with data we reported previously in healthy subjects [21]. After 6 months of treatment, plasma 3-nitrotyrosine was significantly decreased by 82% compared with baseline in the rosiglitazone-treated group (53.1 ± 16 vs 11.7 ± 4 μmol/mol tyrosine, $P < .05$), whereas, in the glyburide-treated group, it increased by 37% as compared with baseline (69.2 ± 18 vs 95 ± 22 μmol/mol tyrosine). There was a highly significant difference between groups in plasma 3-nitrotyrosine after 6 months ($P < .01$) despite similar glycemic control (Fig. 1).

3.3. Inflammation, endothelial function, and adiponectin

At baseline, there were no significant differences in the plasma levels of the inflammatory marker hsCRP, vWF, or adiponectin between the 2 patient groups. Rosiglitazone significantly decreased plasma hsCRP levels by 51% (4.7 ± 4.9 to 2.3 ± 2.6 mg/dL, $P < .05$) and vWF antigen levels by 17% (129% ± 29% to 107% ± 19%, $P < .05$) and significantly increased plasma adiponectin levels by 41% (8.2 ± 3.3 to 11.6 ± 4 μg/mL, $P < .05$) as compared with baseline. Compared with baseline, no statistically significant ($P > .05$) changes in hsCRP (4.2 ± 4.4 vs 3.4 ± 3.5 mg/dL), vWF antigen (145% ± 60% vs 132% ± 58%), or plasma adiponectin levels (9.5 ± 5 vs 9.7 ± 4.2 μg/mL) were observed with glyburide at the 6-month visit.

3.4. Effects of intervention on MBF regulation

At baseline, resting MBF did not differ significantly between subject groups. In the rosiglitazone-treated group, resting perfusion remained unchanged at 6 months (Table 2). In contrast, myocardial perfusion declined by approximately

Table 2
Effects of rosiglitazone and glyburide on MBF

Study group	RMBF (mL/[min g])		SMBF (mL/[min g])		MFR	
	Baseline	6 mo	Baseline	6 mo	Baseline	6 mo
Rosiglitazone	0.9 ± 0.3	0.9 ± 0.2	1 ± 0.3	0.9 ± 0.2	1.1 ± 0.2	1.1 ± 0.2
Glyburide	0.8 ± 0.2	0.7 ± 0.1*,†	1 ± 0.2	0.8 ± 0.2*	1.3 ± 0.3	1.3 ± 0.5

Resting, stress, and myocardial blood flow reserve were measured at baseline and study end as described in "Subjects and methods." Data are expressed as mean ± 1 SD. RMBF indicates resting myocardial blood flow; SMBF, stress myocardial blood flow; MFR, myocardial blood flow reserve (stress/rest flow).

* P less than .05 vs baseline.

† P less than .05 vs rosiglitazone at 6 months.

10% ($P < .05$) in glyburide-treated subjects to levels that were significantly lower than those in subjects treated with rosiglitazone ($P < .05$). In concert, MBF during CPT did not change after 6 months of rosiglitazone treatment, but declined by 24% ($P < .05$) with glyburide therapy ($P < .05$). The MBF reserve was impaired in both subject groups at baseline compared with our previously reported data in healthy nondiabetic subjects [19] and remained unchanged after 6 months of therapy with either agent (Table 2).

Heart rate and rate-pressure product consistently increased during the CPT, with no significant differences between baseline and follow-up studies occurring (data not shown).

3.5. Adverse events

Three subjects in the glyburide group had a mild hypoglycemic event, but none required assistance from another person. However, 2 of these subjects discontinued their assigned treatment and were placed on glipizide for the duration of the study. Analysis of the data excluding these subjects did not significantly effect the results. No patients in the rosiglitazone group reported hypoglycemia. Three subjects in the rosiglitazone arm and 2 in the glyburide arm withdrew for personal reasons unrelated to the study. One subject in the rosiglitazone group reported chest discomfort during the study, and these symptoms were subsequently investigated and not thought to be consistent with myocardial ischemia.

4. Discussion

Cardiovascular disease is the leading cause of death in T2DM [1-3], and multiple hyperglycemia-associated mechanisms are involved including increased nitrosative stress and inflammation [2,9,15,22]. The short-term impact of oral antihyperglycemic therapy on myocardial perfusion and myocardial ischemia is controversial and poorly studied [17,17,23,24]. Here we provide evidence that rosiglitazone, but not glyburide, ameliorated markers of nitrosative stress and inflammation in subjects with T2DM without impairing myocardial perfusion.

The effect of rosiglitazone on myocardial perfusion after 6 months of therapy has recently become the focus of considerable debate and concern [16,24]. A recent meta-analysis of 42 individual, double-blinded, randomized, controlled studies associated the use of rosiglitazone with an increased risk of myocardial infarction or death from cardiovascular causes [16]. All but 4 of these studies were 6 months in duration and, therefore, implicated an early effect of rosiglitazone on myocardial perfusion, a postulate that has led to the addition of myocardial ischemia in a revised label for rosiglitazone. However, longer-term studies in both prediabetic [25] and diabetic patients [24,26,27] failed to confirm an increased risk of myocardial infarction or death attributable to rosiglitazone. The lack of effect of rosiglitazone on myocardial perfusion at rest or during CPT reported herein is consistent with another report in subjects with T2DM in which PET failed to identify an effect of pioglitazone on resting or adenosine-stimulated MBF after 3 months of therapy [28]. In contrast, a nonrandomized 3-month study in healthy T2DM subjects reported that both rosiglitazone and troglitazone corrected deficits of CPT-induced MBF [5]. Finally, a single study using adenosine triphosphate stress thallium 201 scintigraphy reported a beneficial effect of troglitazone on coronary circulation [29]. Neither the study reported here nor these previous reports included subjects with overt coronary vessel disease; and thus, the effects of thiazolidinediones on MBF regulation in subjects with CVD remain unclear.

Glyburide reduced myocardial perfusion both at rest and during CPT. To our knowledge, this is the first report that has studied the effect of oral glyburide on myocardial perfusion in T2DM. Consistent with our studies in T2DM, in catheterized nondiabetic patients, intracoronary glyburide was found to reduce resting coronary blood flow by 9%, increase resting coronary vascular resistance by 15%, and significantly reduce pacing-induced peak coronary peak coronary blood flow [30].

The adenosine triphosphate–sensitive potassium (KATP) channels mediate basal coronary vascular tone in animals and man [30,31]. In the canine coronary circulation, KATP channel blockade with glyburide reduces basal coronary blood flow by up to 52% [31,32]. Activation of KATP channels is thought to be of particular importance in maintaining MBF in subjects with coronary vessel disease [29]. In cardiac surgery patients [32,33], glyburide abolished the protective effect of ischemic preconditioning at rest and in response to metabolic vasodilatation. In contrast, the ability of thiazolidinediones to protect the myocardium

during ischemia and reperfusion remains unclear and potentially species specific [34-37].

Endothelial dysfunction is common in T2DM and may involve the increased production of superoxide anion, which on reaction with nitric oxide can result in the formation of peroxynitrite, a potent oxidant that can impair antioxidant defense systems. Nitrotyrosine, which reflects nitration of tyrosine residues in proteins, is a specific marker for the production of RNS including peroxynitrite [21].

For the first time, we provide evidence that rosiglitazone significantly reduced plasma 3-nitrotyrosine levels, a highly sensitive and specific molecular fingerprint of protein damage by nitrosative stress, to levels that are similar to those reported in healthy nondiabetic subjects [21]. Rosiglitazone also significantly reduced plasma hsCRP and von Willebrand antigen. No changes in these parameters were detected in the patients treated with glyburide despite similar glycemic control. In diabetes, high levels of nitrotyrosine are also associated with myocyte, fibroblast, and endothelial cell apoptosis [38]. Increased immunoreactivity of microvascular nitrotyrosine correlates with glycemic control and levels of intracellular and vascular adhesion molecules [15,39,40].

Oxidative stress has also emerged as a leading candidate in the pathogenesis of changes in acute phase reactants [41]. Our data are consistent with other reports demonstrating that rosiglitazone reduces serum hsCRP by 30% and can decrease reactive oxygen species generation by 40% [42]. Our observations extend these studies because the effect of rosiglitazone on circulating nitrotyrosine has not been reported, suggesting that rosiglitazone may directly regulate processes involved in the development of vascular dysfunction independently of glycemic control. It is however interesting that, despite significant improvements in plasma glucose or in postulated cardiovascular risk biomarkers such as oxidative/nitrosative stress, hsCRP, or adiponectin, there was no significant improvement in the MBF reserve after rosiglitazone treatment.

There are a number of limitations to the data reported herein. First, the number of patients studied is small and may therefore have been insufficient to detect an effect of either treatment on MBF reserve. In addition, HbA_{1c} was higher and resting MBF was slightly lower at baseline in the subjects randomized to glyburide (although neither parameter was significantly different), which may have influenced the results. Finally, more women were randomized to rosiglitazone, which could have also influenced these results.

In summary, our studies indicate that after 6 months of therapy, rosiglitazone reduces nitrosative stress and is without effect on myocardial perfusion at rest or during sympathetic activation in subjects with T2DM without known coronary artery disease. In contrast, glyburide reduces myocardial perfusion equally under both conditions. Our data do not support the contention that rosiglitazone directly contributes to myocardial perfusion deficits during short-term (6 months) treatment.

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