Rosiglitazone Treatment Improves Insulin Regulation and Dyslipidemia in Type 2 Diabetic Cynomolgus Monkeys

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Impairment of peroxisome proliferator-activated receptor-gamma (PPAR-γ), a nuclear receptor that regulates genes involved in lipid and glucose metabolism, may contribute to the onset of metabolic disorders such as diabetes and the accompanying dyslipidemia. Fat-derived tumor necrosis factor alpha (TNF-α) and the acute-phase response protein, C-reactive protein (CRP), may also have a role in the development of obesity-related insulin resistance and type 2 diabetes mellitus. In this study, a group of 14 naturally occurring, insulin-requiring, type 2 diabetic cynomolgus monkeys were used to evaluate the effects of the PPAR-γ agonist, rosiglitazone, on glycemic and lipid parameters and serum levels of TNF-α and CRP. The animals were randomized into 2 groups of 7. One group was treated with 0.5 mg/kg rosiglitazone orally once a day for 7 weeks. Blood was collected for evaluation at baseline, at 2 and 7 weeks during the treatment period, and at 7 and 13 weeks after treatment. Daily insulin requirements were recorded during the entire study. Results showed daily exogenous insulin requirements were significantly reduced ($P < .01$) in those treated with rosiglitazone, while glycemic control was maintained. Plasma triglyceride concentrations were significantly lower ($P < .01$) whereas plasma cholesterol levels tended to be lower and high-density lipoprotein (HDL) concentrations tended to be higher after treatment. No significant differences were noted in TNF-α and CRP serum levels during the treatment period. Body weights remained steady in both groups during the study. These results suggest overall improvement in insulin regulation and lipid profiles during treatment with rosiglitazone.

DIABETES MELLITUS occurs naturally in cynomolgus monkeys (*Macaca fascicularis*) with a clinical presentation similar to that of human type 2 diabetic patients. It often occurs in middle-aged, obese monkeys and begins with insulin resistance, progressing to diabetes, which may require daily exogenous insulin therapy.[1] Other comparable changes are noted in risk markers of cardiovascular disease such as plasma lipid and lipoprotein profiles and also in pathological abnormalities found in the pancreatic islet cells.[1,2] These monkeys are an excellent model for understanding the pathogenesis of type 2 diabetes, as well as determining therapeutic approaches beneficial to diabetic patients.

Peroxisome proliferator-activated receptor-gamma (PPAR-γ) is a nuclear receptor found in the target tissues important to insulin action. It is highly expressed in brown and white adipose tissue and is thought to trigger adipocyte differentiation, promote lipid storage, and modulate the action of insulin.[3] Impairment of PPAR-γ activity may contribute to onset of metabolic disorders such as diabetes and the accompanying dyslipidemia.[3] A class of oral antidiabetic agents, thiazolidinediones, acts as potent agonists of PPAR-γ. Improvement in glycemic control has been noted in patients treated with PPAR-γ agonists either alone or in combination with other antidiabetic agents including insulin.[4,5] Recent evidence also suggests that activation of PPAR-γ improves abnormal lipid concentrations and inhibits early atherosclerotic lesion development in transgenic mouse models of atherosclerosis and diabetes, which may reduce the incidence of cardiovascular disease associated with diabetes.[3,6,7,8]

Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine secreted by a number of cell types, including macrophages and adipocytes. Previous studies have suggested a role for fat-derived TNF-α in the development of obesity-related insulin resistance and type 2 diabetes mellitus.[9] Interestingly, PPAR-γ agonists (thiazolidinediones) have been shown to diminish the synthesis or action of TNF-α in adipocytes in vitro[10] and in adipose tissue in insulin-resistant rodents.[10,11] Another recent report showed that treatment of obese, type 2 diabetic human patients with troglitazone, a PPAR-γ agonist, significantly reduced plasma levels of TNF-α.[12]

C-reactive protein (CRP) is an acute-phase response protein synthesized by the liver.[13] Circulating serum levels of CRP are elevated in response to many infectious or inflammatory diseases and, in particular, elevated CRP may be a reliable predictor of diabetes and cardiovascular disease risk.[14,15] Several studies have correlated CRP with insulin resistance and obesity, which may suggest a role for CRP in the progression and development of type 2 diabetes and associated cardiovascular disease.[16-18] Previous studies with PPAR-γ agonists have identified an anti-inflammatory role for these agents either by direct transcriptional regulation of cytokines and chemokines or through secondary effects of improved metabolism.[19-21] A recent study revealed a reduction in serum levels of CRP in type 2 diabetic patients treated with rosiglitazone.[22]

The purpose of this study was to evaluate the effects of treatment with the PPAR-γ agonist, rosiglitazone, in a group of naturally occurring, insulin-requiring, type 2 diabetic cynomolgus macaques. Exogenous insulin requirements, glycemic measures, plasma lipid profiles, and markers of inflammation were evaluated during 7 weeks of treatment with rosiglitazone.

MATERIALS AND METHODS

Ten male and 4 female type 2 diabetic cynomolgus macaques were single- or pair-housed and fed a standard monkey chow diet (5038, Lab

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Submitted October 8, 2003; accepted March 7, 2004.

Supported in part by a grant from Eli Lilly (J.W.) and Grant No. RR07009 (M.G.) from the National Center for Research Resources.

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0026-0495/04/5309-0002$30.00/0

doi:10.1016/j.metabol.2004.03.014

diet, Purina Mills, Richmond, IN). The animals were maintained with Humulin 70/30 insulin (Eli Lilly, Indianapolis, IN) by intramuscular injections twice per day. A glucometer was used to evaluate blood glucose concentrations, at least twice per week, and exogenous insulin dosages were adjusted to maintain a target blood glucose range of 90 to 125 mg/dL. Daily exogenous insulin requirements were recorded for each animal throughout the study. Values are reported as group means, based on the weekly average of insulin per day for each animal.

The animals were stratified into 2 groups of 7, based on gender, insulin requirements, and plasma triglyceride concentrations. One group was treated orally with rosiglitazone (GlaxoSmithKline, Research Triangle Park, NC) softened in a banana, at 0.5mg/kg once per day for 7 weeks, while control animals received banana alone. This dosage was selected based on previous experience with rosiglitazone in non-human primates (unpublished data).

All of the monkeys were sedated after an overnight fast, with an intramuscular injection of 10 mg/kg ketamine hydrochloride (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) for blood collection and to assess body weight, at 2 baseline time points (2 weeks apart) and at 2 and 7 weeks during the treatment phase and at 7 and 13 weeks after treatment was stopped.

Insulin tolerance tests (ITT) were performed to assess insulin sensitivity at the end of the treatment phase and after 13 weeks of washout.[23] For ITTs, 3-mL blood samples were collected in sodium fluoride sample tubes, at 2 baseline time points and at 3, 6, 9, 12, 15, and 20 minutes after intravenous administration of 0.2 U/kg of regular insulin. Insulin was measured with an enzyme-linked immunosorbent assay (ELISA) kit (Alpco Diagnostics, Windham, NH) and glucose levels were measured by colorimetric assay (Sigma, Diagnostics, St Louis, MO). The area under the curve (AUC) and K value were determined for both glucose and insulin clearance as described previously.[24]

Plasma levels of total cholesterol, triglycerides, and high-density lipoproteins (HDL) cholesterol were determined by enzymatic reaction using a Cobas Fara II analyzer (Roche Diagnostic Systems, Somerville, NJ). Fructosamine levels were determined from plasma separated in tubes containing sodium fluoride, by a colorimetric assay, based on the ability of glycated plasma proteins to reduce nitroblue tetrazolium (Sigma Diagnostics).

Serum levels of TNF-α were measured using a commercial human ELISA kit (Pharmingen, San Diego, CA) according to the manufacturer's instructions, except that 200 μL of monkey serum was used for each assay rather than 100 μL as directed and calculations were adjusted accordingly by dividing the final concentration in half. Serum levels of CRP were measured by competitive ELISA as described by Macy et al.[25]

The data were analyzed for statistical differences using analysis of variance (ANOVA) and covariance (ANCOVA) with repeated measures, or paired *t* test. Significant differences were determined based on a *P* value of less than .05. Data are presented as change in group means over time.

All animal procedures were performed in accordance with the state and federal laws of the US Department of Health and Human Services and within the guidelines established by the Institutional Animal Care and Use Committee.

RESULTS

No significant change was noted in body weight between the 2 groups of monkeys throughout the treatment period. Mean body weight at baseline for control animals was 8.59 kg $\pm$ 1.16 and for rosiglitazone-treated animals was 8.55 kg $\pm$ 1.32. Mean body weight after 7 weeks treatment for control animals was 8.58 kg $\pm$ 1.20 and for rosiglitazone-treated animals was 8.50 kg $\pm$ 1.4. The drug was well tolerated and no signs indicating

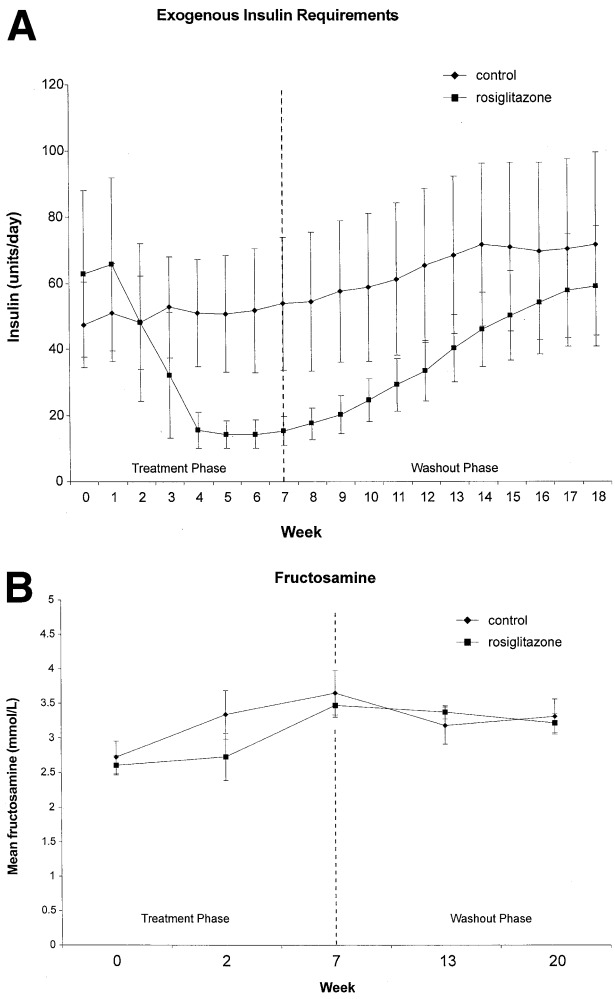

Fig 1. (A) Daily exogenous insulin requirements expressed as weekly averages for each group. The reduction noted in rosiglitazone-treated animals is statistically significant (ANCOVA, *P* < .01). (B) Maintenance of glycemic control as measured by fructosamine levels for each group during the course of the study. Error bars represent SEM.

fluid retention such as pendulous or facial edema or other adverse effects of the drug were evident throughout the study.

A significant reduction in exogenous insulin requirement was noted in the animals during treatment with rosiglitazone (repeated-measures ANCOVA, $P < .01$) (Fig 1A), which returned to baseline amounts after the 13-week washout period. Both groups maintained glycemic control throughout the study, as determined by fructosamine levels (Fig 1B). However, there was a slight, but significant increase noted in the fructosamine levels in both groups during the treatment phase of the study, as analyzed by paired *t* test ($P < .05$ control, $P < .01$ treatment). Insulin sensitivity, as determined by the increase in glucose clearance during the ITT, was significantly better during treatment with rosiglitazone compared to the washout period (K values: control animals during treatment 2.53 $\pm$ 0.63 and washout 2.24 $\pm$ 0.27; rosiglitazone-treated animals during treatment 2.58 $\pm$ 0.33 and washout 1.41 $\pm$ 0.20; *t* test, $P < .01$) (Fig 2).

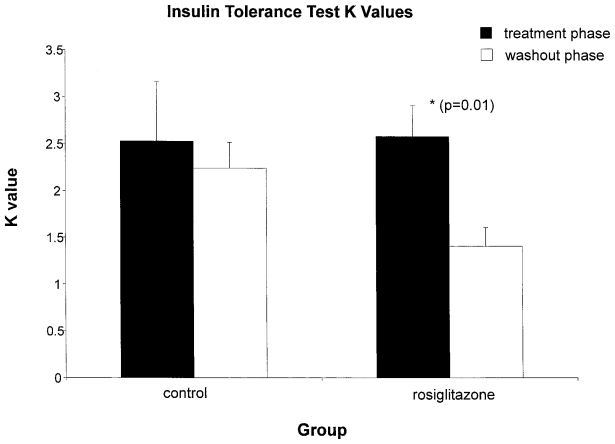

Fig 2. Calculated K values for glucose clearance during insulin tolerance testing. The change noted in rosiglitazone-treated monkeys is statistically significant (*t* test, $P < .01$). Error bars represent SEM.

A significant decrease in plasma triglyceride levels was noted from baseline through 7 weeks of treatment with rosiglitazone (repeated-measures ANCOVA, $P < 0.01$) (Table 1). A decrease in total plasma cholesterol and an increase in HDL-cholesterol were noted during the treatment period, but these trends were not significant. Lipid and lipoprotein measures returned to baseline levels following the 13-week washout phase.

No statistically significant difference was detected in serum TNF-α or CRP levels for either group. TNF-α level for the control group at baseline was 0.75 ± 0.27 pg/mL and after treatment was 1.22 ± 0.36 pg/mL, and for rosiglitazone-treated animals at baseline was 1.63 ± 0.23 pg/mL and after treatment was 1.93 ± 0.62 pg/mL. Mean absolute change in TNF-α levels from baseline through the 7-week treatment period for control animals was 0.477 ± 0.24 pg/mL and for the rosiglitazone-treated animals was 0.299 ± 0.42 pg/mL. CRP level for the control group at baseline was 0.422 ± 0.08 mg/L and after treatment was 0.392 ± 0.061 mg/L, and for rosiglitazone-treated animals at baseline was 0.382 ± 0.13mg/L and after treatment was 0.318 ± 0.11. The mean absolute change in CRP levels from baseline through the treatment period for the control animals was −0.031 ± 0.031 mg/L and for rosiglitazone-treated animals was −0.064 ± 0.036 mg/L.

DISCUSSION

The use of thiazolidinediones to treat type 2 diabetes is becoming more common. While their effectiveness at improving insulin sensitivity has been proven, there are still many questions remaining as to the mechanisms involved. This study used a previously described animal model of diabetes[1,2] to evaluate the effects of rosiglitazone. It also serves to further characterize this animal model and enhance its contribution to future studies in the field.

No clinical signs of illness or adverse effects of the drug were apparent during this study. The monkeys appeared healthy and maintained their body condition throughout the study as evidenced by the steady body weights recorded. This differs from previous reports, which have demonstrated an increase in body weight in human patients treated with thiazolidinediones,[26,27] as well as similar reports of weight gain in rodent models treated with rosiglitazone.[28,29] This finding may be attributed to the shorter duration of treatment during this study compared to many of the human trials or to the simultaneous reduction in required exogenous insulin while on treatment with rosiglitazone, since insulin therapy has been shown to contribute to weight gain.[27,28,30] Additionally, the monkeys in this study were fed in a manner that limited hyperphagia, a side effect found in both human patients and rodent models treated with thiazolidinediones, which likely contributes to weight gain.[28,31]

Similar to reports in human patients, rosiglitazone significantly improved insulin sensitivity in the diabetic monkeys. This is evidenced by the reduction in exogenous insulin required while maintaining glycemic control similar to the non-treated monkeys (Fig 1A and B). All monkeys maintained fructosamine levels within a normal range for insulin-maintained diabetic monkeys, although there was a notable increase in both groups (Fig 1B). This is likely a reflection of the numerous experimental manipulations during the study that required sedation of the animals and then necessitated less rigorous insulin treatment to avoid hypoglycemic accidents. In addition, insulin sensitivity, as determined by ITT, was better during the treatment phase as compared to the washout phase for the rosiglitazone-treated group, whereas no change was detected in the control group (Fig 2).

Cynomolgus monkeys with type 2 diabetes present with a dsylipidemic profile similar to that of human diabetics, making cardiovascular disease risk studies relatable to this model. Specifically, these animals have elevated triglyceride levels (mean at baseline, 368 mg/dL; normal, 30 to 50 mg/dL) and lower than normal HDL-cholesterol (mean at baseline, 40 mg/dL; normal, 49 mg/dL).[32] Rosiglitazone treatment improved these parameters, especially triglyceride levels, which were

Table 1. Plasma Lipid and Lipoprotein Values for Each Group at Baseline and at 2 and 7 Weeks of Treatment With Rosiglitazone and After 13 Weeks of Washout

	Control	Rosiglitazone
Triglycerides (mg/dL)		
Week 0-baseline	478.14 ± 163.50	259.43 ± 60.22
Week 2-treatment	461.71 ± 150.23	78.43 ± 11.74*
Week 7-treatment	512.86 ± 138.70	128.57 ± 15.09*
Week 13-washout	462.43 ± 103.85	296.33 ± 40.60
Cholesterol (mg/dL)		
Week 0-baseline	189.57 ± 29.09	165.86 ± 18.32
Week 2-treatment	175.71 ± 29.15	134.71 ± 11.06
Week 7-treatment	174.14 ± 25.04	140.14 ± 10.54
Week 13-washout	152.86 ± 16.75	145.57 ± 10.56
HDL-cholesterol (mg/dL)		
Week 0-baseline	40.57 ± 6.67	41.00 ± 3.54
Week 2-treatment	44.14 ± 10.33	58.57 ± 5.80
Week 7-treatment	35.86 ± 6.71	46.43 ± 3.13
Week 13-washout	33.14 ± 5.16	42.14 ± 4.09

NOTE. Values are expressed as means ± SEM.
*Repeated-measures ANCOVA, $P < .01$.

significantly decreased during the treatment period (Table 1). There was also a tendency toward lowering of total plasma cholesterol and increasing HDL-cholesterol. These findings are somewhat different from those reported in human studies: most have not shown a significant change in triglyceride or plasma cholesterol levels during treatment with rosiglitazone either alone or in combination with insulin.[5,33] However, at least one study did show a significant increase in HDL-cholesterol levels during treatment with rosiglitazone,[34] and another reported a significant improvement in triglyceride levels during treatment with insulin and troglitazone.[27] The dose of rosiglitazone used in these animals is higher than that commonly used to treat human type 2 diabetic patients[21,22,26] and some differences noted between our study and human trials may be attributed to this variable.

No statistically significant changes were noted in TNF-α or CRP levels in this study. This is likely due to a limitation in statistical power with the small number of animals available and the sensitivity of the assays to detect a difference. Additionally, the length of the study may not have been long enough for significant changes in these markers to become apparent. Interestingly, a slight increase was noted in serum levels of TNF-α in both groups of animals during the treatment phase of the study. The cause for this increase is unclear. The importance of this to our study is that animals treated with rosiglitazone had less of an increase in TNF-α serum levels, suggesting an effect of the drug on stimulated TNF-α. A mild reduction was noted in CRP serum levels in both groups during the study and this trend was more evident after 7 weeks of treatment with rosiglitazone. These findings are similar to results reported in humans and rats as previously mentioned,[11,12,22] but further evaluation and larger study groups will be required to make claims as to mechanism and interactions of TNF-α, CRP, insulin resistance, and PPAR-γ agonists.

In conclusion, the results of the current study indicate overall improvement in glycemic control and lipid parameters in type 2 diabetic cynomolgus monkeys during treatment with rosiglitazone without affecting body weight. No significant change could be detected in the markers of inflammation, TNF-α and CRP, during this study.

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