A Selective Peroxisome Proliferator-Activated Receptor α Agonist, CP-900691, Improves Plasma Lipids, Lipoproteins, and Glycemic Control in Diabetic Monkeys


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ABSTRACT

Peroxisome proliferator-activated receptors (PPARs) are involved in the regulation of lipid and glucose metabolism. PPARα agonists improve insulin sensitivity and hyperglycemia and are effective in treating type 2 diabetes mellitus (T2DM), whereas PPARα agonists are used to treat dyslipidemia and atherosclerosis. The goal here was to examine the efficacy of a selective PPARα agonist ([S]-3-[3-(1-carboxy-1-methyl-ethoxy)-phenyl]-piperidine-1-carboxylic acid 4-trifluoromethyl-benzyl ester; CP-900691) on lipid, glycemic, and inflammation indices in 14 macaque monkeys with spontaneous T2DM maintained on daily insulin therapy. Monkeys were dosed orally with either vehicle (n = 7) or CP-900691 (3 mg/kg, n = 7) daily for 6 weeks. CP-900691 treatment increased plasma high-density lipoprotein cholesterol (HDLc) (33 ± 3 to 60 ± 4 mg/dL, p < 0.001) and apolipoprotein A1 (96 ± 5 to 157 ± 5 mg/dL, p < 0.001), reduced plasma triglycerides (547 ± 102 to 356 ± 90 mg/dL, p < 0.01), and apolipoprotein B (62 ± 3 to 45 ± 3 mg/dL, p < 0.01), improved the lipoprotein index (HDLc to non-HDLc ratio; 0.28 ± 0.06 to 0.79 ± 0.16, p < 0.001), decreased body weight (p < 0.01) and C-reactive protein (CRP) (1700 ± 382 to 304 ± 102 ng/ml, p < 0.01), and increased adiponectin (1697 ± 542 to 4242 ± 1070 ng/ml, p < 0.001) compared with baseline. CP-900691 treatment reduced exogenous insulin requirements by approximately 25% (p < 0.04) while lowering plasma fructosamine from 2.87 ± 0.9 to 2.22 ± 0.17 mM (p < 0.05), indicative of improved glycemic control. There were no changes in any of the aforementioned parameters in the vehicle group. Because low HDLc and high triglycerides are well established risk factors for cardiovascular disease, the marked improvements in these parameters, and in glycemic control, body weight, and CRP, suggest that CP-900691 may be of benefit in diabetic and obese or hyperlipidemic populations.

The peroxisome proliferator-activated receptors (PPARα, PPARβ, and PPARγ) are a family of distinct nuclear receptors that associate with key naturally occurring lipid molecules and function as transcription factors in the regulation of carbohydrate and lipid metabolism, among other biological processes (Harwood and Hamanaka, 1998; Corton et al., 2000; Willson et al., 2000; Winegar et al., 2001). The PPARs became recognized as important pharmaceutical targets for the treatment of dyslipidemia, diabetes, and the cardiovascular disorders associated with obesity and the metabolic syndrome, when it was discovered that PPARγ was the therapeutic target for the marketed glitazone class of antidiabetic agents (Willson et al., 2000, 2001; Picard and Auwerx, 2002) and PPARα was the therapeutic target for the marketed fibrate class of antidyshlipidemic agents (Harwood and Hamanaka, 1998; Willson et al., 2000; Winegar et al., 2001). Because the pharmacological actions of PPARα, PPARβ, and PPARγ activators are distinct (Corton et al., 2000; Willson et

ABBREVIATIONS: PPAR, peroxisomal proliferator-activated receptor; CP-900691, (S)-3-[3-(1-carboxy-1-methyl-ethoxy)-phenyl]-piperidine-1-carboxylic acid 4-trifluoromethyl-benzyl ester; T2DM, type 2 diabetes mellitus; HDLc, high-density lipoprotein; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDLC, high-density lipoprotein cholesterol; apoA1, apolipoprotein A1; apoB, apolipoprotein B; ITT, insulin tolerance test; CRP, C-reactive protein; ANOVA, analysis of variance; TG, triglyceride; ELISA, enzyme-linked immunosorbent assay.
PPARα, which is highly expressed in liver, skeletal muscle, and heart and activated by a variety of naturally occurring lipids and the fibrate class of dyslipidemic drugs, potentiates fatty acid oxidation and modulates lipoprotein metabolism (Corton et al., 2000; Jones, 2001; Picard and Auwerx, 2002). Fibrates such as fenofibrate, for example, have been shown in both laboratory and clinical studies to reduce plasma triglyceride (TG) levels, reduce the number of circulating triglyceride-rich apolipoprotein B (apoB)-containing very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) particles, increase high-density lipoprotein cholesterol (HDL-C) levels through in-creases in apolipoprotein A1 (apoA1) production, and favor-ably affect atherosclerotic progression and cardiovascular disease (Harwood and Hamanaka, 1998; Corton et al., 2000; Jones, 2001; Castrillo and Tontonoz, 2004; Li et al., 2000, 2004). Recent clinical studies evaluating chronic treatment with the PPARα activators fenofibrate and bezafibrate have indeed demonstrated improvement in total, HDL, and LDL cholesterol and triglycerides, and corresponding reductions in atherosclerotic progression (Diabetes Atherosclerosis Intervention Investigators, 2001) and improved cardiovascular outcomes (Elkeles et al., 1998).

Though primarily involved in lipid metabolism, PPARα may also provide a link between dyslipidemia and diabetes, because T2DM patients generally also present with a variety of dyslipidemias, such as increased TG and circulating fatty acids and decreased HDLC, which further predispose them to cardiovascular disease (Willson et al., 2000). In insulin-resistant rodents, fibrates have been shown to decrease adiposity and improve insulin sensitivity, attenuating both hyperinsulinemia and hyperglycemia (Guerre-Millo et al., 2000). In addition, the PPARα agonist fenofibrate has been shown to lower plasma TG and LDL cholesterol concentra-tions, increase plasma HDLC, and ameliorate hyperinsulinemia in obese rhesus monkeys (Winegar et al., 2001) and elevate HDLC levels in vervet monkeys (Wallace et al., 2005). However, supraphysiological glucose levels in vitro and hyperglycemia in vivo have been shown to attenuate PPARα mRNA expression in β-cells and PPARα expression in islets, respectively, and may negatively affect PPARα-related β-cell lipid oxidation (Roduit et al., 2000). Furthermore, fenofibrate is not specific for PPARα activation and shows a similar degree of PPARγ activation (Willson et al., 2000; Jones, 2001), thus confounding interpretation of the role of PPARα agonism in glycemic regulation.

Similar to human T2DM patients, diabetic cynomolgus monkeys exhibit increased plasma concentrations of TG and VLDL cholesterol and decreased HDLC concentrations (Wagner et al., 2006), all of which may be favorably altered by PPARα activation. To determine whether pharmacologic PPARα activation by highly specific PPARα agonists would lead to improved insulin sensitivity and exhibit favorable effects on dyslipidemia, we have examined the effects of a highly selective PPARα agonist, (S)-3-[3-(1-carboxy-1-methyl-ethoxy)-phenyl]-piperidine-1-carboxylic acid 4-trifluoromethyl-benzyl ester (CP-900691) (Hayward et al., 2006), on lipid and glycemic indices in this clinically relevant animal model of diabetic dyslipidemia.

Materials and Methods

Characteristics of CP-900691. CP-900691 (Fig. 1) is a speci-fic, saturable, high-affinity ligand for PPARα, as demonstrated in scintillation-proximity competition ligand binding assays using recombinant human PPARα, PPARβ, and PPARγ receptor sub-type ligand binding domains, where IC50 values for PPARα, PPARβ, and PPARγ competition with standard radiolabeled ligands averaged 12.5 ± 0.4, >10,000, and 1520 ± 170 nM, respectively. The 122-fold specificity relative to PPARγ binding and apparent lack of binding of CP-900691 to PPARβ was paralleled by 61- and 207-fold specificities relative to PPARα and PPARγ activation, respectively (PPARα EC50 = 22 nM, PPARβ EC50 = 4548 nM, and PPARγ EC50 = 1776 nM), in cell-based functional assays of PPAR activation (Hayward et al., 2006). Dose Selection and Study Parameters. In selecting a dose for evaluating the actions of CP-900691 in diabetic monkeys, we com-pared the pharmacokinetic parameters in other species where CP-900691 reduced plasma TG in mice with an ED50 of 0.12 mg/kg, reduced plasma TG and apoB-containing lipoprotein (VLDL + LDL) cholesterol in obese dogs with an ED50 of 1 mg/kg, and increased plasma HDLC in mice by 2.5-fold at a dose of 1 mg/kg (Hayward et al., 2006). When pharmacokinetic parameters were assessed in the lean monkeys after a single 1 mg/kg intravenous dose and after a single 1 mg/kg oral dose, CP-900691 exhibited an oral bioavailability of 16%, a Cmax of 610 ng/ml at the Tmax of 1.8 h, a half-life of 3.8 h, a Cl of 15 ml/min/kg, and a Vd of 1.7 L/kg. Because the bioavail-ability in the monkey was less than that observed in the rat and dog (16% versus 39 and 59%, respectively) and the clearance was higher in the monkey than in the rat or dog (15 versus 2.7 and 0.3 ml/min/kg, respectively), we chose to study once-daily oral administration of 3 mg/kg/day CP-900691 for 6 weeks, an oral dose three times higher than the dose that showed efficacy in the dog studies.

Animal Protocol. Fourteen cynomolgus monkeys (Macaca fascicularis) with naturally occurring T2DM (Wagner et al., 2006) were included in the study. All animals were fed a standard monkey chow diet (Purina Lab Diet, Richmond, IN), divided into two meals per day, and received twice-daily insulin injections (70% intermediate-acting (e.g., NPH insulin), 30% short-acting (i.e., regular insulin)). Insulin doses were based on postprandial blood glucose measurements determined with a glucometer (from tail stick of conscious animals). Blood glucose concentrations were monitored by this method twice weekly, or more often if necessary, to achieve a postprandial blood glucose concentration of 100 to 200 mg/dl. Exogenous insulin requirements were recorded daily and averaged weekly for each mon- key. All experimental procedures involving animals in this study were conducted in accordance with Public Health Service policy and approved by and comply with the guidelines of the Institutional Animal Care and Use Committee of Wake Forest University Health Sciences.

Monkeys were stratified into two treatment groups based, in order of priority, on sex, body weight, average daily exogenous insulin requirements, and plasma HDLC and TG concentrations determined from blood samples collected 8 weeks before dosing. Parameters (mean ± S.E.M.) for vehicle control versus drug treatment groups,

Fig. 1. The structure of CP-900691.
respectively, were as follows: plasma TG, 182 ± 57 versus 220 ± 49 mg/dl; total cholesterol, 145 ± 11 versus 186 ± 41 mg/dl; HDLC, 43.7 ± 3.9 versus 38.4 ± 4.1 mg/dl; apoA1, 114 ± 7 versus 115 ± 9 mg/dl; apoB, 36 ± 5 versus 50 ± 5 mg/dl; glucose, 251 ± 27 versus 255 ± 38 mg/dl; fructosamine, 2.32 ± 0.16 versus 2.74 ± 0.14 mM; C-peptide, 0.41 ± 0.11 versus 0.53 ± 0.15 pm; exogenous insulin requirement, 89 ± 18 versus 98 ± 21 IU/day; and body weight, 8.6 ± 1.2 versus 10.3 ± 0.8 kg. No significant group differences were present.

Two sets of baseline measurements were collected 4 and 2 weeks before dosing, followed by 6 weeks of daily dosing with either the PPARD agonist CP-900691 at a dose of 3 mg/kg/day (n = 7) or its vehicle (n = 7). At the conclusion of dosing, the CP-900691 animals underwent 4 weeks of washout, and the control group entered into a reduced-dose crossover paradigm where the animals that received vehicle for the initial 6 weeks of dosing received 3 weeks of daily dosing with CP-900691 at the 10-fold reduced dose of 0.3 mg/kg/day.

All treatment animals were dosed orally with either vehicle or CP-900691 once a day with their afternoon feeding. The vehicle consisted of a mixture of 0.5% methocel and 0.1% Polysorbate 80. Fresh CP-900691 dosing solution was prepared each day at a concentration of 100 mg/ml. The compound was weighed and added to the vehicle, vortexed for 10 s or until thoroughly wetted, and then stirred magnetically for at least 1 h before dosing. Vehicle or drug was absorbed into a Primatreat, a piece of fruit, or other palatable treat and consumed voluntarily by each animal.

To record body weight and collect blood samples for measurement of glycemic and lipid indices, monkeys were sedated (after a 16-h fast and exogenous insulin withdrawal) with ketamine (10–15 mg/kg i.m.), followed by supplemental maintenance doses (3–5 mg/kg i.m.) as necessary. Samples were taken at baseline, after every 2 weeks of dosing, and then 4 weeks after cessation of dosing (washout, only in animals receiving the test compound). Whole blood samples in EDTA-treated tubes were placed on ice immediately after collection and centrifuged, and the resulting plasma was removed and stored at −80°C.

Insulin tolerance tests (ITTs) were performed at week −4 (baseline) and week 4 of dosing, as described previously (Gee et al., 2004). Two sets of baseline blood samples (−5 min and 0 min) were collected. After drawing the second baseline blood sample, regular insulin was infused at a dose of 0.2 IU/kg (0.5 ml/kg). Blood samples were subsequently collected at 3, 6, 9, 12, 15, and 20 min postinsulin. All blood samples were centrifuged, and plasma was stored at −80°C for batched analysis. Parameters measured include areas under the curve for glucose and insulin and K values for glucose disappearance rate.

Clinical Chemistry Analyses. Total plasma cholesterol was measured by enzymatic techniques based on the methods of Allain et al. (1974). Plasma TG were determined by the methods of Fossati and Prencipe (1982). HDLC concentrations were measured by using a slight modification (2 M MnCl2) of the heparin-manganese precipitation method described in the Manual of Laboratory Operations of the National Heart, Lung, and Blood Institute Lipid Research Clinics Program (National Heart, Lung, and Blood Institute, 1974). All analyses were performed on an ACE Alera Clinical Chemistry System (Alfa Wasserman, Inc., West Caldwell NJ). ApoA1, apoB, and apoCIII levels were determined by specific enzyme-linked immunoassay kit (ELISA) as described previously (Chandler et al., 2003; Harwood et al., 2003). The lipoprotein index was calculated as the ratio of HDLC to apoB-containing lipoprotein (VLDL + LDL) cholesterol (Gotto et al., 1995).

Plasma glucose and fructosamine concentrations were assayed by enzymatic colorimetric methods using the new glucose UV and FRUC kit (Roche Diagnostics, Indianapolis, IN). The interassay and intra-assay coefficients of variation were <5% for glucose and <10% for fructosamine. Insulin, c-peptide, and adiponectin were determined by using ELISA kits (Merodia, Uppsala, Sweden), with <10% interassay and intra-assay coefficients of variation. Plasma C-reactive protein (CRP) was measured by using ELISA kits from ALPCO Diagnostics (Salem, NH).

Statistical Analysis. All results are reported as mean values ± S.E.M. Statistical analyses were performed by using Statistica 6 (StatSoft, Tulsa, OK). Log transformation of variables was performed when normality assumptions were not met. Baseline values for lipid and glycemic variables are the average of two separate samples collected 2 and 4 weeks before dosing. Intergroup comparisons at baseline were performed by using one-way ANOVA. Comparisons of glycemic and lipid variables at subsequent time points were made by using repeated measures ANOVA. ITT parameters at 4 weeks of treatment were analyzed by analysis of covariance, with baseline values serving as covariates. Tukey HSD post hoc tests were performed to identify specific intragroup or intergroup differences when ANOVA/analysis of covariance p < 0.05.

Results

A comparison of the mean baseline values of the lipid and glycemic parameters and of CRP and adiponectin for the vehicle-treated and CP-900691-treated cohorts at the beginning of the study is shown in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle-Treated</th>
<th>CP-900691-Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>249 ± 53</td>
<td>547 ± 144</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>139 ± 8</td>
<td>169 ± 20</td>
</tr>
<tr>
<td>LDL + VLDL cholesterol</td>
<td>61 ± 4</td>
<td>78 ± 8</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>56 ± 4</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>109 ± 8</td>
<td>96 ± 8</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>41 ± 4</td>
<td>62 ± 3</td>
</tr>
<tr>
<td>Apolipoprotein CIII</td>
<td>2.5 ± 0.5</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Glycemic indices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>239 ± 30</td>
<td>257 ± 35</td>
</tr>
<tr>
<td>Fructosamine (mM)</td>
<td>2.30 ± 0.13</td>
<td>2.87 ± 0.13</td>
</tr>
<tr>
<td>C-peptide (pM)</td>
<td>0.40 ± 0.08</td>
<td>0.54 ± 0.17</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>139 ± 43</td>
<td>134 ± 49</td>
</tr>
<tr>
<td>Inflammation markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (ng/ml)</td>
<td>1839 ± 610</td>
<td>1700 ± 382</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>1011 ± 283</td>
<td>1697 ± 542</td>
</tr>
<tr>
<td>Exogenous insulin (IU)</td>
<td>92 ± 29</td>
<td>113 ± 30</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>8.7 ± 1.2</td>
<td>10.6 ± 0.8</td>
</tr>
</tbody>
</table>

TABLE 1

Baseline lipid and glycemic parameters

Blood samples for baseline blood measures were collected at −2 and −4 weeks before commencement of dosing as outlined under Materials and Methods. Data are the mean of the average baseline values for each parameter ± S.E.M.
and plasma apoB-containing lipoprotein cholesterol levels in response to treatment with CP-900691, total plasma cholesterol was unchanged by treatment with either vehicle or CP-900691 (data not shown). The absence of a notable change in total cholesterol concentration even though plasma LDL and VLDL cholesterol were markedly reduced was a consequence of a dramatic, up to 2.2-fold, increase in plasma HDLC concentration (Fig. 3A), which increased from 33 ± 3 to 65 ± 3 mg/dL after only 2 weeks of dosing with CP-900691 and remained similarly elevated throughout the 6-week dosing interval (*p < 0.05 versus baseline), with no change in plasma HDLC levels in the vehicle-treated group.

Because the HDL-elevating effect of PPARα agonism by fibrates is caused, in part, by receptor-mediated up-regulation of apoA1 production (Harwood and Hamanaka, 1998; Willson et al., 2000), we evaluated the effects of CP-900691 on plasma apoA1 concentrations. Similar to the effects of CP-900691 treatment on HDLC levels, plasma apoA1 concentrations were also increased after CP-900691 treatment relative to baseline and vehicle controls after 2 weeks of dosing and remained significantly elevated throughout the course of the study (Fig. 3B). Plasma HDLC and plasma apoA1 levels both returned to baseline values within 4 weeks of cessation of treatment with CP-900691.

The lipoprotein index is commonly used to assess the potential cardiovascular risk associated with various distributions of cholesterol between the lipoprotein subclasses among individuals where a high lipoprotein index is considered more favorable than a lower lipoprotein index (Gotto et al., 1995). As shown in Fig. 4, the lipoprotein index remained constant at approximately 0.6 throughout the course of the study in the vehicle-treated cohort, but increased approximately 2.5-fold within the first 2 weeks of treatment with CP-900691 and remained elevated at that level throughout the 6 weeks of treatment. The lipoprotein index returned to near background within 4 weeks of cessation of treatment, consistent with the time course of return to baseline of plasma TG, apoB-containing lipoproteins, and HDLC concentrations (see above).

Changes in Insulin Requirements and Glycemic Measures. Insulin requirements for monkeys in the vehicle group remained unchanged throughout the 6-week study period, whereas daily insulin needs in the CP-900691 monkeys declined significantly by the 3rd week of

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**Fig. 2.** Reduction of plasma triglycerides and ApoB by CP-900691. Plasma triglycerides and apoB levels were measured at baseline, after 2, 4, and 6 weeks of treatment with CP-900691, and 4 weeks after cessation of treatment (washout; WO) as described under Materials and Methods. Shown are percentage of baseline plasma triglycerides (A) and apoB concentrations (B) for vehicle-treated animals (○) and animals treated with 3 mg/kg day CP-900691 (■) as a function of duration of administration. Data are expressed as mean percentage of average baseline values ± S.E.M.
dosing and remained markedly diminished (p < 0.05 versus baseline) at every time point from weeks 3 through 6 of treatment (Fig. 5). After cessation of treatment, the rebound in insulin requirement was slow relative to the return to baseline observed for the lipid parameters (see above). Indeed, the requirement for exogenous insulin continued to decline during the first 2 weeks of washout and thereafter increased by only 10% from the nadir mean insulin requirement noted 1 week after cessation of treatment.

To demonstrate the accuracy with which adjustments in exogenous insulin requirements were made temporally during the course of treatment with CP-900691, plasma fructosamine concentrations were measured. Similar to the use of glycated hemoglobin levels as a marker of long-term glycemic status, changes in plasma fructosamine levels are a sensitive marker of changes in short-term glycemic control (Cefalu et al., 1993). Figure 6 illustrates a time-dependent reduction in fructosamine levels in the CP-900691-treated cohort such that plasma fructosamine levels were significantly lower than baseline (p < 0.05) after 6 weeks of treatment. These observations, together with the sustained reduction in exogenous insulin requirements, are indicative of a long-term improvement in glycemic control by treatment with CP-900691.

There were no differences in fasting glucose or insulin levels between the vehicle- and CP-900691-treated cohorts (Table 2). This is likely caused by the monitoring and adjustment in exogenous insulin administration throughout the course of study. In addition, there were no differences in mean C-peptide levels between control and treated cohorts throughout the course of the study (Table 2), consistent with the reduced capacity for glucose-stimulated insulin secretion in these animals (Wagner et al., 2006).

To determine whether the reductions in exogenous insulin requirements and fructosamine concentrations were the result of improvements in insulin sensitivity resulting from treatment with CP-900691, ITTs were conducted at baseline and after 4 weeks of treatment. Calculated areas under the curves for ITT at each time point, along with the respective $K_g$ values for glucose disappearance rate, are presented in Table 3. CP-900691 did not alter areas under either the glucose or insulin curves compared with vehicle-treated controls. Similarly, treatment with CP-900691 did not significantly alter the slopes of the glucose disappearance curves ($K_g$) compared with either $K_g$ values obtained at baseline or $K_g$ values ob-

![Fig. 3. Elevation of plasma HDLC and ApoA1 by CP-900691. Plasma HDLC and apoA1 levels were measured at baseline, after 2, 4, and 6 weeks of treatment with CP-900691, and 4 weeks after cessation of treatment (washout; WO) as described under Materials and Methods. Shown are percentage of baseline HDLC (A) and apoA1 concentrations (B) for vehicle-treated animals (□) and animals treated with 3 mg/kg day CP-900691 (■) as a function of duration of administration. Data are expressed as mean percentage of average baseline values ± S.E.M.](https://jpet.aspetjournals.org/cover.jpg)
The percentage of average baseline values as a function of duration of drug administration. Data are expressed as mean and animals treated with 3 mg/kg day CP-900691 as a function of duration of drug administration. Data are expressed as mean percentage of average baseline values ± S.E.M. Shown are percentage of baseline lipoprotein indices for vehicle-treated animals and animals treated with 3 mg/kg day CP-900691 as a function of duration of drug administration. Data are expressed as mean percentage of average baseline values ± S.E.M.

**Fig. 4.** Improvement in lipoprotein index by CP-900691. The lipoprotein index, which is the ratio of plasma HDLC to plasma LDL + VLDL cholesterol concentration, was calculated from the plasma VLDL + LDL, and HDL cholesterol concentrations measured at baseline, after 2, 4, and 6 weeks of treatment with CP-900691, and 4 weeks after cessation of treatment (washout; WO) as described under Materials and Methods. Shown are percentage of baseline lipoprotein indices for vehicle-treated animals (■) and animals treated with 3 mg/kg day CP-900691 (■) as a function of duration of drug administration. Data are expressed as mean percentage of average baseline values ± S.E.M.

**Fig. 5.** Reduction in requirement for exogenous insulin by CP-900691. The amount of exogenous insulin required to maintain plasma glucose levels at baseline values was recorded daily and averaged weekly for each animal throughout the course of the study as outlined under Materials and Methods. Shown are the average daily exogenous insulin requirements for the vehicle-treated animals (□) and animals treated with 3 mg/kg day CP-900691 (■) as a function of duration of drug administration. Data are expressed as mean daily exogenous insulin unit requirements ± S.E.M.

**Fig. 6.** Improvement in glycemic control by CP-900691. Plasma fructosamine, a marker of short-term antecedent glycemic control, was measured at baseline, after 2, 4, and 6 weeks of treatment with CP-900691, and 4 weeks after cessation of treatment (washout; WO) as described under Materials and Methods. Shown are percentage of baseline plasma fructosamine concentrations for vehicle-treated animals (□) and animals treated with 3 mg/kg day CP-900691 (■) as a function of duration of administration. Data are expressed as mean percentage of average baseline values ± S.E.M.

**Body Weight Reduction.** There was no significant difference in average body weight between the vehicle- and CP-900691 treated animals at baseline (8.7 ± 1.0 versus 10.6 ± 0.6 kg, p = 0.21). Although food consumption was observed to be normal for all animals, there was a significant, time-dependent reduction in overall body weight in the animals receiving CP-900691 treatment relative to baseline measurements (Fig. 7), with CP-900691 treatment leading to significant reductions after 4 weeks (−4.4 ± 1.3%) and 6 weeks (−5.7 ± 1.5%) of treatment (both p < 0.05 versus baseline). Body weight remained unchanged in the vehicle group over the course of the study. In addition, similar to the slow rebound of exogenous insulin requirements after cessation of CP-900691 treatment (see above), mean body weight in the CP-900691 cohort continued to decline slightly for the first 4 weeks of washout and did not return to baseline within the duration of the study.

**Improved CRP and Adiponectin.** Biomarkers of inflammation were improved with plasma CRP concentrations reduced by 68% after 2 weeks of treatment with CP-900691 and sustained reductions throughout the 6 weeks of treatment with a return to baseline after 4 weeks of washout (Fig. 8A). In contrast, plasma adiponectin, a fat-derived hormone, produced in an inverse relationship with the amount of fat stores (Picard and Auwerx, 2002), was increased by 55% after 2 weeks of treatment with CP-900691 and continued to increase to over 300% of baseline after 6 weeks of treatment, before returning toward baseline values after 4 weeks of washout (Fig. 8B).

**Reduced Dose Crossover Study.** The observation that the highly selective PPARγ agonist CP-900691, at 3 mg/kg, favorably affected glycemic control in addition to its beneficial effects on the dyslipidemia in these diabetic monkeys, suggests that PPARγ-specific receptor agonism may be capable of favorably affecting glycemic control independent of concomitant PPARγ agonism. Although CP-900691 demonstrated an approximately 122-fold specificity for PPARγ over PPARα, the peak plasma concentration of CP-900691 in the diabetic monkeys at the 3.0 mg/kg dose evaluated (1.31 nM) was only slightly less than the IC50 values obtained in the competition binding assay for CP-900691 association with PPARγ (1.52 nM) and the EC50 values obtained for PPARγ functional agonism of CP-900691 in cultured cells (1.78 nM). This suggests that at least for a short time after each dose there was sufficient CP-900691 in the plasma of the treated monkeys to partially activate PPARγ.

To determine whether partial, short-term PPARγ agonism was responsible for the improvements in glycemic control reported above, at the conclusion of the above study, animals...
ITT parameters

Table 3

<table>
<thead>
<tr>
<th>Glucose (mg/dl)</th>
<th>Baseline</th>
<th>Treatment Effects at 2 Weeks</th>
<th>Treatment Effects at 4 Weeks</th>
<th>Treatment Effects at 6 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n = 7)</td>
<td>239 ± 30</td>
<td>186 ± 41</td>
<td>185 ± 31</td>
<td>221 ± 38</td>
</tr>
<tr>
<td>CP-900691 (n = 7)</td>
<td>257 ± 35</td>
<td>181 ± 52</td>
<td>186 ± 37</td>
<td>246 ± 41</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>Vehicle</td>
<td>139 ± 43</td>
<td>117 ± 35</td>
<td>165 ± 49</td>
</tr>
<tr>
<td>CP-900691</td>
<td>130 ± 49</td>
<td>118 ± 45</td>
<td>128 ± 47</td>
<td>123 ± 52</td>
</tr>
<tr>
<td>C-peptide (ng/ml)</td>
<td>Vehicle</td>
<td>0.40 ± 0.08</td>
<td>0.33 ± 0.07</td>
<td>0.54 ± 0.27</td>
</tr>
<tr>
<td>CP-900691</td>
<td>0.54 ± 0.17</td>
<td>0.23 ± 0.07</td>
<td>0.47 ± 0.14</td>
<td>0.74 ± 0.32</td>
</tr>
</tbody>
</table>

Fasted plasma glucose, insulin, and C-peptide concentrations were measured at baseline and every 2 weeks during dosing, as outlined under Materials and Methods. Data are expressed as mean values ± S.E.M.

**Discussion**

The hallmark of PPARα agonism is its ability to reduce plasma triglycerides and apoB-containing lipoproteins (VLDL and LDL) and elevate plasma HDLC concentrations (Gotto et al., 1995; Harwood and Hamanaka, 1998; Faergeman, 2000; Willson et al., 2000). For this reason fibrates have broad appeal for treating a variety of dyslipidemias, including diabetic dyslipidemia (Gotto et al., 1995; Harwood and Hamanaka, 1998; Willson et al., 2000). Although several fibrates have shown improvements in insulin sensitivity and glycemic control in addition to benefits on dyslipidemia (Guerre-Millo et al., 2000; Willson et al., 2000; Winegar et al., 2001), these fibrates show little specificity for activation of PPARα relative to activation of PPARγ (Harwood and Hamanaka, 1998; Corton et al., 2000; Willson et al., 2000), which precludes an estimation of the impact of PPARα agonism on glycemic control per se.

To evaluate the effects of PPARα agonism on insulin sensitivity and glycemic control we studied the actions of a highly selective PPARα agonist, CP-900691, on the lipid and glycemic indices of T2DM monkeys.

CP-900691 demonstrated effects consistent with PPARα agonism. In vitro, CP-900691 has 120-fold specificity for PPARα relative to PPARγ and a more than 10,000-fold specificity relative to PPARβ, with respect to both competition ligand binding and functional receptor activation (Hayward et al., 2006). Reported here, CP-900691 markedly reduced plasma triglycerides and apoB, reduced apoB-containing lipoproteins, markedly elevated HDLC and apoA1 concentrations, and improved the lipoprotein index (HDL to non-HDLC ratio). CRP, a sensitive proinflammatory biomarker, was also decreased. These beneficial changes all occurred rapidly, demonstrating maximal effects within 2 weeks of treatment and maintaining achieved levels of efficacy throughout the 6-week course of study, similar to the time course of efficacy onset noted in clinics for beneficial effects of fibrates on dyslipidemia (Corton et al., 2000; Willson et al., 2000; Jones, 2001; Castrillo and Tontonoz, 2004). Furthermore, the lipid, lipoprotein, and CRP changes induced by CP-900691 all returned to baseline within 4 weeks after
cessation of treatment, indicative of a primary effect of the compound on these parameters.

CP-900691 also improved glycemic control. Unlike the rapid onset of maximal changes and rapid return to baseline after cessation of treatment in plasma lipoproteins and CRP, the reduction in exogenous insulin requirements in response to CP-900691 treatment was slower in onset and sustained even after cessation of treatment. In addition to the decrease in insulin requirements, there was a significant reduction in plasma fructosamine, a surrogate indicator of glycemic control (Cefalu et al., 1993). Similarly, adiponectin, a marker of reduced adipose mass, reduced inflammation, and improved insulin sensitivity (Fernandez-Real et al., 2003; Tan et al., 2004), increased across the 6-week treatment period, also consistent with a secondary effect of CP-900691 action. The improvements in glycemic status, however, were not a direct consequence of increased peripheral insulin sensitivity, because the rate of glucose disappearance from ITTs, which is mediated primarily by muscle, was not different between control and CP-900691-treated animals. Effects on hepatic insulin sensitiv-

**TABLE 4**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>After 3-Week Treatment</th>
<th>Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>279 ± 41</td>
<td>171 ± 23</td>
<td>−39</td>
<td>0.05</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>48.7 ± 4.6</td>
<td>60.3 ± 5.5</td>
<td>+24</td>
<td>0.008</td>
</tr>
<tr>
<td>Exogenous insulin (IU)</td>
<td>101 ± 33</td>
<td>78 ± 35</td>
<td>−23</td>
<td>0.02</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>1730 ± 433</td>
<td>2362 ± 559</td>
<td>+37</td>
<td>0.006</td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>1763 ± 467</td>
<td>567 ± 207</td>
<td>−68</td>
<td>0.02</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>8.73 ± 1.24</td>
<td>8.42 ± 1.23</td>
<td>−3.6</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Fig. 8.** Changes in CRP and adiponectin by CP-900691. Plasma CRP concentrations (A), a marker of systemic inflammation, and adiponectin (B), a marker of inflammation and insulin sensitivity, were measured at baseline, after 2, 4, and 6 weeks of treatment with CP-900691, and 4 weeks after cessation of treatment (washout; WO) as described under Materials and Methods. Shown are percentage of baseline for vehicle-treated animals (○) and animals treated with 3 mg/kg day CP-900691 ( ■) as a function of duration of administration. Data are expressed as mean percentage of average baseline values ± S.E.M.
ity were not determined and may likely have been improved secondarily to the improved hepatic lipoprotein metabolism.

In addition to benefits on dyslipidemia and glycemic control, CP-900691 significantly reduced overall body weight. The reduction in body weight followed a time course similar to that noted for reduction in exogenous insulin requirement, exhibiting a slow onset, a steady decline through the course of study, and a continual decrease even after treatment cessation. Like the requirements for exogenous insulin, the slow onset and sustained reduction in body weight were suggestive of secondary effects of PPARα agonism.

Whether the reduction in insulin requirement and concomitant reduction in insulin-mediated weight gain was responsible for the overall body weight reduction noted in these animals or whether a PPARα-agonism-mediated reduction in body weight was responsible for the reduced insulin requirement of these animals is uncertain. However, it is clear from the reduced dose crossover data and the lack of weight gain in the CP-900691-treated animals [weight gain is a hallmark of PPARγ activation in clinic (Willson et al., 2000; Picard and Auwerx, 2002) and no weight reduction was found in T2DM monkeys treated with the PPARγ-specific agonist, rosiglitazone (Gee et al., 2004)], that these effects are likely mediated primarily through PPARα agonism rather than through PPARγ agonism, because effects persisted even when the dose of CP-900691 was reduced to a level where the potential for concomitant PPARγ activation was negligible (C_{max} levels 11.8-fold lower than the EC_{50} for PPARγ activation).

Changes in markers of inflammation were also improved in these monkeys, consistent with other studies suggesting that PPARα agonism also reduces the proinflammatory state associated with diabetes, obesity, and cardiometabolic syndrome (Willson et al., 2000; Li et al., 2004). Reductions in CRP occurred rapidly, demonstrating maximal effects within 2 weeks of treatment and maintaining achieved levels of efficacy throughout the 6-week treatment. By contrast, adiponectin concentrations exhibited a steady increase throughout the study. These observations are in stark contrast to alterations in CRP and adiponectin noted in these animals after treatment with the PPARγ-specific agonist, rosiglitazone, where adiponectin levels were rapidly elevated to maximal levels within 2 weeks of treatment and were maintained at those elevated levels for the remainder of treatment, and where CRP levels were only marginally decreased (Gee et al., 2004; Wagner et al., 2006). Furthermore, when the dose of CP-900691 was reduced, decreases in CRP and increases in adiponectin after CP-900691 treatment persisted further, suggesting that these effects are likely both mediated primarily through selective PPARα agonism.

The differential effects of CP-900691 and rosiglitazone on CRP and adiponectin may also serve to underscore fundamental differences in the actions of PPARα and PPARγ agonism. For example, a central role of the adipose tissue in the effects of PPARγ-activating drugs, such as rosiglitazone, on glucose homeostasis has been well established (Picard and Auwerx, 2002). The rapid and sustained increase with rosiglitazone treatment of adiponectin, a cytokine of adipocyte origin whose gene is under the positive control of a PPARγ response element (Combs et al., 2002), is not surprising and is likely a primary effect of the drug. By contrast, the primary site of action of PPARα agonists, like CP-900691, is the liver (Corton et al., 2000; Willson et al., 2000), and it is believed that increases in adiponectin produced by PPARα agonists occur secondary to reduced weight loss and adipose tissue mass and likely improved hepatic insulin action (Yang et al., 2001). The pattern of adiponectin increase observed after CP-900691 treatment, which developed slowly relative to changes in lipid metabolism and CRP levels and more closely paralleled the weight reduction induced by the compound, is consistent with this hypothesis.

The rapid and sustained decrease of CRP in response to CP-900691 treatment, which paralleled the time course of modulation of lipid metabolism, is not surprising and is likely a primary effect of the drug because CRP is produced by the liver and is under direct negative control by PPARα agonists (Willson et al., 2000). By contrast, changes in CRP in response to PPARγ agonism occur as a secondary response to reductions in tumor necrosis factor α, an adipocyte-derived signaling molecule (Picard and Auwerx, 2002) that controls CRP expression in the liver and whose expression is blunted by PPARγ agonists (Willson et al., 2000; Picard and Auwerx, 2002; Gee et al., 2004). That rosiglitazone, the highly selective PPARγ agonist, showed no changes in tumor necrosis factor α and only small reductions (approximately 20–25%) in CRP (Gee et al., 2004; Wagner et al., 2006) is consistent with the secondary nature of this action.

The differences in the responses of CRP and adiponectin to CP-900691 and rosiglitazone treatment further support the suggestion that the actions of CP-900691 reported here are primarily the result of PPARα activation by the compound rather than a composite of concomitant PPARα and PPARγ activation. However, it should be noted that the possibility exists for altered PPARγ sensitivity to drug actions or elevated drug concentrations to occur in specific target tissues that could potentially result in PPARγ activation in those tissues at plasma drug concentrations that would theoretically preclude PPARγ-mediated contributions to efficacy. Therefore, the role of PPARγ activation in the favorable actions of CP-900691 on glycemic control cannot be entirely ruled out. Studies in knockout mouse models would be needed to definitively address this.

In conclusion, one unifying aspect of the pharmacology of the plethora of PPAR agonists that have been developed over the past decade that has remained a verity is that each agonist exhibits a distinct profile of activation of the three classes of PPARs, and as a consequence, each exhibits a somewhat distinct efficacy profile with respect to modulation of lipid and carbohydrate metabolism and a distinct safety profile. In this article, we describe the efficacy profile of a highly PPARα-specific PPAR agonist that demonstrates favorable effects on dyslipidemia and glycemic control, body weight, and inflammation in T2DM monkeys. Whether such an efficacy profile for CP-900691 would translate to human T2DM patients with associated diabetic dyslipidemia and whether similar efficacy would also be observed in obese nondiabetic patients or patients with cardiometabolic syndrome remains to be determined.

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