A novel PPARα agonist ameliorates insulin resistance in dogs fed a high-fat diet

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Tsunoda M, Kobayashi N, Ide T, Utsumi M, Nagasawa M, Murakami K. A novel PPARα agonist ameliorates insulin resistance in dogs fed a high-fat diet. Am J Physiol Endocrinol Metab 294: E833–E840, 2008. First published January 22, 2008; doi:10.1152/ajpendo.00627.2007.—Agonism of peroxisome proliferator-activated receptor (PPAR) α, a key regulator of lipid metabolism, leads to amelioration of lipid abnormalities in dyslipidemic patients. However, whether PPARα agonism is an effective form of therapy for obesity-related insulin resistance associated with lipid abnormalities is unclear. The present study investigated the effects of a potent and subtype-selective PPARα agonist, KRP-101, in a nonrodent insulin-resistant animal model under pair-fed conditions. Beagle dogs were fed a high-fat diet for 24 wk to induce insulin resistance. During the final 12 wk, 0.03 mg·kg⁻¹·day⁻¹ KRP-101 (n = 5) or vehicle (n = 5) was administered orally once a day. KRP-101 administration resulted in a significantly lower weight of overall visceral fat, which is associated with increased adiponectin and decreased leptin in serum. KRP-101 administration improved hyperglycemia and hyperinsulinemia as well as dyslipidemia in dogs fed a high-fat diet. Oral glucose tolerance test showed that KRP-101 administration improved glucose intolerance. The KRP-101 group showed a markedly lower hepatic triglyceride concentration. Lipid oxidation was increased in the liver and skeletal muscles of the KRP-101 group. These findings in the dog model suggest that the use of potent and subtype-selective PPARα agonists as a potentially relevant therapeutic approach to treat human insulin resistance associated with visceral obesity.

KRP-101; obesity; insulin resistance; muscle lipid oxidation

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)α is a member of the nuclear receptor superfamily and plays a pivotal role in lipid metabolism (36). PPARα is expressed more abundantly in organs that have a high lipid catabolic activity, such as the liver, skeletal muscle, heart, kidney, and brown adipose tissues (6). Fibrates, drugs widely used to treat dyslipidemic subjects, are able to activate PPARα (19). PPARα activation by fibrates is thought to be responsible, at least in part, for their triglyceride lowering and high-density lipoprotein (HDL)-cholesterol increasing effects (40).

A number of rodent studies demonstrated that PPARα agonists have the ability to improve insulin resistance as well as dyslipidemia (4, 24, 27). Concomitantly, it has been shown that PPARα agonists prevent increases in adiposity and body weight in rodent disease models without reducing food intake (15). Fibrates often improve glucose intolerance in type 2 diabetic patients (16, 23). However, the recent Fenofibrate Intervention and Event Lowering in Diabetes study in a larger number of subjects did not consistently show a pronounced effect on glucose levels in type 2 diabetic patients (20). There are no obvious clinical effects of fibrates on adiposity or body weight.

It has been suggested that differences in the efficacy of fibrates against insulin resistance and obesity between rodents and humans can be explained as follows. First, rodents show markedly higher levels of brown adipose tissue than nonrodents, including adult humans (26). Therefore, the appetite-independent antiobesity effect reported in rodent studies may be attributable to effects mainly in brown adipose tissues. In fact, PPARα agonists strongly induced lipid oxidation-associated energy expenditure in the brown adipose tissues of rodents (2). Second, the expression level of PPARα in the liver is at least 10-fold higher in rodents than in nonrodents (30, 31). Therefore, rodent studies could overestimate the pharmacological effects of PPARα agonists in humans. Third, we cannot exclude the possibility that PPARα agonism by fibrates was insufficient in clinical studies because of the weak PPARα agonistic activity of these agents (7).

Interestingly, in nonrodent studies, fenofibrate was reported to improve glucose intolerance as well as dyslipidemia in dogs fed a high-fat diet and in obese rhesus monkeys, although there were no significant antiobesity effects (38, 43). More recently, it was reported that K-111, a more potent PPARα agonist than fibrates, suppressed obesity with a concomitant reduction in food intake (5). Thus, even in nonrodent studies, it has not yet been clarified whether PPARα agonists have an antiobesity effect through independent mechanisms of reducing food intake.

Previous studies demonstrated that the dog is a further example of a species in which neonatal brown adipose tissue is replaced in adulthood by a tissue with morphological and biochemical characteristics of adipose tissue similar to that in humans (1, 17). In the present study, a high-fat diet was given to dogs as a nonrodent animal model to induce obesity-associated insulin resistance. Using this model, we examined the therapeutic effects of a potent PPARα agonist under pair-fed conditions. This study investigated whether a highly potent and subtype-selective PPARα agonist can suppress insulin resistance and obesity without affecting food intake in obese and insulin-resistant dogs.

MATERIALS AND METHODS

Compounds. (2S)-2-[[3-(4-fluorophenoxy)phenyl]methyl]carbamoyl)-4-methoxyphenyl)methyl] butanoic acid (KRP-101) was synthesized by Kyorin Pharmaceutical Co., Ltd. KRP-101 has been

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reported to be a potent and subtype-selective PPARα agonist in a Gal4-transactivation assay using human PPAR subtypes (29). Fenofibr acid was purchased from Tyger Scientific (Ewing, NJ). The compounds were dissolved in dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO), and the final concentrations of DMSO were set at 0.1% in transactivation assay.

**Plasmid.** cDNAs encoding the ligand-binding domains (LBD) of human, dog, and rat PPARα, PPARγ, and PPARβ were obtained from cDNA libraries by PCR. These cDNAs were inserted in the pM vector (Clontech, Palo Alto, CA) including the DNA-binding domain (DBD) of Gal4, a yeast transcriptional factor, to prepare pM-PPARs, which were expression vector plasmids for Gal4 DBD/PPAR LBD fusion proteins. pFR-Luc, a firefly luciferase reporter plasmid, and pRL-Luc (pRL-TK), a Renilla luciferase internal standard plasmid, were purchased from Stratagene (La Jolla, CA) and Promega (Madison, WI), respectively.

**Transactivation assay.** CHO-K1 cells were purchased from Dainippon Pharmaceutical (Osaka, Japan). Cells were seeded at 5 × 104 cells/well in 24-well plates and cultured with Ham’s F-12 medium supplemented with 10% FCS overnight in a 5% CO2–95% air incubator set at 37°C. Cells were cotransfected with 40 ng/well of pM-PPARs, 400 ng/well of pFR-Luc, and 5 ng/well of pRL-TK using Lipofectamine (Invitrogen, Carlsbad, CA) for 2 h. Transfected cells were treated with various concentrations of the compound for 20 h. Cells were washed with PBS and harvested using Passive Lysis Buffer (Promega). Firefly and Renilla luciferase activities were determined using the Dual Luciferase Reporter 1000 Assay System (Promega), and luminescence was measured with a LUCY2 Microplate Luminescence Reader (Anthos, Salzburg, Austria). The luciferase activity of each sample was defined as firefly luciferase activity normalized by Renilla luciferase activity.

**Administration.** A solution of KRP-101 was prepared at a concentration of 0.3 mg/ml in 10 mmol/l sodium bicarbonate containing 2 mmol/l sodium hydroxide (vehicle solution). The dose was set at 0.1 ml/kg. After 12 wk on a high-fat diet, the solution of KRP-101 (0.03 mg·kg−1·day−1) in a gelatin capsule (Torpac, Fairfield, NJ) was orally administered to the animals as described above. Vehicular solution in a gelatin capsule was orally administered to control animals fed a high-fat diet once a day at a dosage of 0.03 mg·kg−1·day−1 for 12 wk. Blood was drawn from the mediceps vein to determine serum biochemical parameters. At the end of the administration period, the dogs were initially anesthetized by intramuscular injection of ketamine (10 mg/kg), and surgical anesthesia was maintained with additional doses of intravenous pentobarbital sodium (25–40 mg/kg). The liver, skeletal muscle, and white adipose tissues were immediately excised from animals killed by exsanguination under anesthesia.

**Serum biochemical parameters.** Triglyceride levels were determined using Liquitex TG II reagents (Roche Diagnostics, Tokyo, Japan). Total cholesterol levels were determined using a Cholesterol E-test Wako kit (Wako Pure Chemical Industries, Osaka, Japan). Free fatty acid levels were determined using an NEFA C-test Wako kit (Wako Pure Chemical Industries). HDL cholesterol levels were determined using a Cholesterol E-test Wako kit and HDL cholesterol precipitation reagents (Wako Pure Chemical Industries). Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. Serum insulin was determined by a sandwich enzyme immunoassay method using dog insulin standard (Morinaga Bioscience, Yokohama, Japan) and an insulin assay kit (Morinaga Bioscience). The primary antibody was determined to have >100% cross-reactivity to dog insulin. The operating range of the assay was established as 78 to 5,000 pg/ml using dog insulin standard. Serum adiponectin was determined using a mouse/rat adiponectin ELISA kit.

### Table 1. Primer sequences for RT-QPCR analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
<th>Probe</th>
</tr>
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<tbody>
<tr>
<td>CPT1A</td>
<td>CGGGGCCCCCTGACT</td>
<td>CAAAATAAGGGTGGGCAGACCTTG</td>
<td>CGGAGGCTACTCTTTCC</td>
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<tr>
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<td>CCCCAGCGAGCAAGAAAGG</td>
<td>CATCCGCGAGCAAGAAAGG</td>
<td>CAGCGCGGAGATCCAG</td>
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<tr>
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<td>CTCAGACGTTGAAAGTAGGA</td>
<td>ACAACAAGGCTCCATCTCC</td>
</tr>
<tr>
<td>TBP</td>
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<td>ACCATCTCTTGGCCAAGAAATCA</td>
<td>CGGAGGCTAGTTCTGG</td>
</tr>
</tbody>
</table>

RT-QPCR, real-time quantitative PCR; CPT1A, carnitine palmitoyltransferase 1A; VLCAD, very long-chain acyl-CoA dehydrogenase; TBP, TATA-binding protein.

### Table 2. EC50 values of KRP-101 showing agonistic activities for human, dog, and rat PPARα, PPARγ, and PPARβ

<table>
<thead>
<tr>
<th>Species</th>
<th>PPARα EC50 value, mmol/l</th>
<th>PPARγ EC50 value, mmol/l</th>
<th>PPARβ EC50 value, mmol/l</th>
</tr>
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<tbody>
<tr>
<td>Human</td>
<td>12 ± 2</td>
<td>1,500 ± 50</td>
<td>1,650 ± 100</td>
</tr>
<tr>
<td>Dog</td>
<td>12 ± 2</td>
<td>2,970 ± 30</td>
<td>1,550 ± 100</td>
</tr>
<tr>
<td>Rat</td>
<td>12,000 ± 1,200</td>
<td>3,200 ± 200</td>
<td>&gt;10,000</td>
</tr>
</tbody>
</table>

Data are shown as means ± SE of three independent experiments. PPARα, peroxisome proliferator-activated receptor. The cDNAs encoding the ligand-binding domain (LBD) of PPARα, PPARγ, and PPARβ were inserted in the pM vector containing the DNA-binding domain of Gal4. Gal4-fused PPAR LBD plasmids were cotransfected into CHO-K1 cells with Gal4-responsive reporter plasmids and internal standard plasmids. Transfected cells were treated with various concentrations of KRP-101 for 20 h.
The primary antibody was rabbit polyclonal antibody against full-length mouse adiponectin. The anti-mouse adiponectin antibody is known to have cross-reactivity with serum dog adiponectin. The intra- and interassay coefficients of variation in the adiponectin assay using dog serum were 8.5 and 7.5%, respectively. Leptin level was determined by RIA methods using a multi-species leptin RIA kit (Linco Research, St. Charles, MO).

**Fig. 1.** Concentration-response curves of KRP-101 (closed circles) and fenofibric acid (open circles), a major active metabolite of fenofibrate, on transcriptional activity for dog peroxisome proliferator-activated receptor (PPAR) α. The cDNA encoding the ligand-binding domain (LBD) of PPARα was inserted in the pM vector containing the DNA-binding domain (DBD) of Gal4. Gal4-fused PPARα LBD plasmid was cotransfected into CHO-K1 cells with Gal4-responsive reporter plasmid and internal standard plasmid. Transfected cells were treated with various concentrations of KRP-101 or fenofibric acid for 20 h. Data are shown as means ± SE of three independent experiments.

**Fig. 2.** Biochemical characteristics of dogs fed a high-fat diet. High-fat diets were given to dogs for 12 wk. Body weight (A), serum levels of triglyceride (B), free fatty acid (C), total cholesterol (D), non-high-density lipoprotein (HDL) cholesterol (E), glucose (F), insulin (G), leptin (H), and adiponectin (I) in dogs were measured in the fed state. The results are shown as means ± SE; n = 12. *P < 0.05, significantly different vs. value before feeding a high-fat diet (wk 0).

**Oral glucose tolerance test.** To better clarify the effect of KRP-101 on glucose intolerance, a separate experiment using dogs fed a high-fat diet was performed. KRP-101 (0.03 mg/kg; n = 3) or vehicle (n = 3) was administered orally to dogs fed a high-fat diet for 3 wk. An oral glucose tolerance test (OGTT) was carried out after 2 wk of drug administration. Dogs were loaded with glucose solution at 1 g/kg after overnight fasting. Blood was drawn from the medicephalic vein at 0, 30, 60, and 120 min after glucose loading. Values for area under the curve from 0–120 min (AUC0–120 min) for insulin and glucose levels were calculated from the values at 0, 30, 60, and 120 min after glucose loading. Insulin sensitivity index was evaluated as 10,000/ square root of (fasting glucose concentration × fasting insulin concentration) × (mean glucose concentration × mean insulin concentration during OGTT) (28).

**Tissue lipids.** Lipid contents of the liver and muscle were extracted according to the method of Folch et al. (12) with some modifications. Tissues were homogenized in 2:1 (vol/vol) chloroform-methanol, the homogenates were extracted using metabolic shakers for 1 h and centrifuged at 3,000 rpm for 15 min, and the supernatant was evaporated to dryness. The tissue pellet was extracted two times with 2:1 (vol/vol) chloroform-methanol in the same manner. The extracts were dried and redissolved in 4% Triton X-100 for lipid measurement. Triglycerides were determined using Liquiteck TG II reagents (Roche Diagnostics).

**Fatty acid oxidation.** [1-14C]palmitic acid was obtained from New England Nuclear (Boston, MA). The measurement of fatty acid oxidation was determined by CO2 production from palmitic acid in the liver and muscle as described previously (18). Tissue was incubated in glass vials containing Krebs-Ringer phosphate HEPES buffer (pH 7.4) supplemented with 0.2% BSA and 0.25 mM palmitic acid (0.5 μCi/ml [1-14C]palmitic acid) at 37°C for 2 h. Each glass vial was connected with thick rubber tubing to a scintillation vial, which held a glass filter filled with Soluene 350 (Packard Instrument, Downers Grove, IL) for collecting CO2. After the metabolic reaction was stopped by the addition of HClO4, the samples were left at room temperature to collect 14CO2. Methanol was added to the scintillation vials containing the glass filter before addition of ACSII Scintillation Cocktail (GE Healthcare, Little Chalfont, UK). The radioactivity of
14CO2 was counted with a liquid scintillation counter (Packard Instrument).

Real-time quantitative PCR analysis. We isolated total RNA from the liver and white adipose tissue using an RNeasy mini kit (Qiagen, Valencia, CA). Total RNA was treated with RNase-free DNase and reverse transcribed using random primers (SuperScript II First-Strand Synthesis System; Invitrogen). To prepare the cDNA from the total RNA isolated from the tissues, reverse transcription was performed using 500 ng of the total RNA in 20 μl reaction mixtures containing 1× first-strand buffer, 100 ng of random primers, 0.5 mmol/l dNTP mixture, 10 mmol/l dithiothreitol, 40 units of RNaseOUT, and 200 units of SuperScript II reverse transcriptase (Invitrogen). The cDNA was stored at -20°C until analysis. Real-time quantitative PCR was performed using an ABI Prism 7500 with TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA). TaqMan Universal Master Mix and Target Assay Mix were combined, and aliquots of 9 μl were added to 6 μl of diluted cDNA in 96-well plates. For each reaction, the polymerase was activated by preincubation at 95°C for 10 min. Amplification was then performed by 40 cycles at 95°C for 15 s and 60°C for 60 s. Sequence Detector Software (version 1.2; Applied Biosystems) was used to analyze the PCR data. The real-time PCR data were standardized with the TATA-binding protein gene in white adipose tissues and with the eukaryotic 18S ribosomal RNA gene in liver. Commercially available Assays-on-Demand probes and primer mix (Applied Biosystems) for eukaryotic 18S rRNA were used (Hs99999901_s1) and were designed using the Primer Design program (Applied Biosystems) for carnitine palmitoyltransferase 1A (CPT1A), very long-chain acyl-CoA dehydrogenase (VLCAD), and adiponectin. The primer sequences used are indicated in Table 1.

Data analysis. To determine the EC50 values, nonlinear regression with sigmoidal dose-response analysis was performed. Values are presented as means ± SE. Paired t-test was performed to assess the significance of differences between mean values before feeding a high-fat diet and those after feeding a high-fat diet. Unpaired t-test was performed to assess the significance of differences between pairs of mean values. P < 0.05 was considered significant. All analyses were performed using Prism (GraphPad Software, San Diego, CA).

RESULTS

Effects of KRP-101 on PPAR transactivation. To test the effects of KRP-101 on transcriptional activities for human, dog, and rat PPAR subtypes (PPARα, PPARγ, and PPARδ), we performed transient transfection assays using Gal4 DBD/PPARs LBD chimeras. KRP-101 activated human PPARα, PPARγ, and PPARδ with EC50 values of 13, 2,500, and 1,920 nmol/l, respectively (Table 2). Almost similar EC50 values were obtained from transfection assays using dog PPARs. However, agonistic activity of KRP-101 for rat PPARs was very weak. These findings suggest that KRP-101 has the ability to act as a subtype-selective PPARα agonist in dogs and humans but not in rats.

We next compared agonistic activity for dog PPARα between KRP-101 and fenofibric acid, a major active metabolite of fenofibrate (13). Fenofibric acid activated dog PPARα in a concentration-dependent manner but with a very weak potency (Fig. 1). The EC50 value of fenofibric acid was estimated to be at least >1,250-fold weaker than that of KRP-101.
Effects of KRP-101 on serum biochemical parameters. Beagle dogs were fed a high-fat diet for 12 wk. Twelve weeks of a high-fat diet resulted in increased body weight and elevated serum levels of triglycerides, total cholesterol, non-HDL cholesterol, glucose, insulin, and leptin compared with values before feeding a high-fat diet (Fig. 2, A, B, D, E, F, G, and H). Serum free fatty acids and adiponectin levels were decreased from values before feeding a high-fat diet (Fig. 2, C and I).

At 12 wk, dogs fed a high-fat diet were divided into the following two groups: one (KRP-101: n = 5) received 0.03 mg·kg⁻¹·day⁻¹ of KRP-101 with the high-fat diet and the other received vehicle (control: n = 5) with the high-fat diet. Food intake of the control group was restricted; therefore, there was no change between KRP-101 (489 ± 8 g·animal⁻¹·day⁻¹) and the control (469 ± 19 g·animal⁻¹·day⁻¹) groups. There was no significant difference in body weight changes between the KRP-101 group and the control group (Fig. 3A). As expected, KRP-101 treatment resulted in markedly lower serum levels of lipids, such as triglycerides, free fatty acids, total cholesterol, and non-HDL cholesterol (Fig. 3, B–E). Serum levels of insulin and glucose were decreased in the KRP-101 group compared with controls (Fig. 4, A and B).

To examine the effect of KRP-101 on glucose intolerance in dogs fed a high-fat diet, another set of experiments was performed under pair-fed conditions (Fig. 5). KRP-101 or vehicle was administered to dogs fed a high-fat diet for 3 wk. OGTT was performed after 2 wk of drug treatment. During OGTT, KRP-101 significantly decreased serum insulin levels at all time points of 0, 30, 60, and 120 min after glucose load (Fig. 5A). Consistent data were also obtained from serum insulin AUC₀–120 min (Fig. 5C). Serum glucose AUC₀–120 min was not significantly changed by KRP-101 treatment (Fig. 5D), although there was a significant change only 60 min after glucose load (Fig. 5B). Insulin sensitivity index was significantly increased by KRP-101 treatment (Fig. 5E). After 3 wk of drug treatment, serum parameters were measured in a fed state. Serum insulin level was lower in the KRP-101 group (control: 1.2 ± 0.09 ng/ml; KRP-101: 0.4 ± 0.08 ng/ml, P < 0.05). Serum glucose level (control: 92 ± 3 mg/dl; KRP-101: 87 ± 7 mg/dl, not significant) and body weight (control: 15.3 ± 0.2 kg; KRP-101: 16.2 ± 1.1 kg, not significant) were not significantly different in the two groups.

Effects of KRP-101 on white adipose tissues and serum adipokines. At 12 wk, there was no significant change in body weight between KRP-101 and the control groups. KRP-101
resulted in significant decreases in the weights of visceral (omental, mesenteric, and perirenal) adipose tissues (Table 3). Similarly, KRP-101 tended to decrease subcutaneous adipose tissue weight, but the effect was not significant. There was no difference in liver weight between the two groups. KRP-101 decreased the serum leptin level and increased the adiponectin level compared with the respective levels before feeding a high-fat diet (Fig. 6, A and B). Consistent with the serum adiponectin level, increased adiponectin mRNA in mesenteric white adipose tissue was observed in the KRP-101 group (Fig. 6C).

Effects of KRP-101 on lipid concentrations and lipid catabolic activity in liver and skeletal muscle. To investigate the mechanism underlying the improvement of insulin resistance by KRP-101, lipid concentrations were measured in insulin target tissues, such as the liver and skeletal muscle. KRP-101 inhibited hepatic triglyceride accumulation (Fig. 7A) accompanied by a significant increase in fatty acid oxidation in the liver (Fig. 7C). These effects were accompanied by increases in hepatic mRNA levels of CPT1A and VLCAD, key regulators in fatty acid oxidation (Fig. 8, A and B). In skeletal muscle, no difference was observed between KRP-101 and the control groups in the triglyceride concentration (Fig. 7B) despite the significant increase in fatty acid oxidation in KRP-101 groups (Fig. 7D).
DISCUSSION

Lipid overflow in insulin target tissues is a major cause of insulin resistance induced by Western diets, which is termed by lipotoxicity (8, 9, 35). Activation of PPARα, a key nuclear receptor in lipid metabolism, is involved in the beneficial effects of fibrates on lipid profiles (14). Several clinical trials have shown varying success in reducing cardiovascular events in metabolic syndrome, including insulin resistance (34). Importantly, fibrate therapy has shown a more significant reduction of cardiovascular risk in patients with features of metabolic syndrome than in those without such features (34), raising the possibility that fibrates have other beneficial effects beyond their well-established action on plasma lipids. In the present study, we demonstrated that insulin resistance in dogs fed a high-fat diet was markedly improved by a potent and subtype-selective PPARα agonist, KRP-101, under pair-fed conditions. Moreover, this agonist suppressed increased adiposity accompanied by a decrease in serum leptin and an increase in serum adiponectin. These observations suggest the therapeutic potential of PPARα agonist in insulin resistance in humans.

In a cell-based transactivation assay, KRP-101 was a highly potent and subtype-selective agonist for human and dog PPARα, but not for rat PPARα. It is known that humans and rodents show great species differences in response to PPARα agonist on transactivation assay (41, 42). It has been demonstrated that two amino acid residues in helix 3 of the LBDs of PPARα are major contributors to species differences in response to a PPARα agonist (21, 30). Our cloning data showed that two amino acid residues of dog PPARα are completely conserved to the human type (30). These findings suggest that this compound is a useful tool for investigating the pharmacological profile of PPARα activation in dogs fed a high-fat diet.

KRP-101 significantly improved hyperglycemia and hyperinsulinemia in insulin-resistant dogs. OGTT results strongly suggested improvement of glucose intolerance by KRP-101 treatment. The antidiabetic effect of PPARα activation was also supported by other reports using nonrodent models (4, 24, 27). Lipid accumulation in the liver is associated with several features of insulin resistance and metabolic syndrome in humans (25, 37). Improvement of insulin resistance by KRP-101 may be explained by reduction of increased triglyceride concentration and increases in lipid oxidation in liver, because elimination of lipotoxicity could be responsible for amelioration of insulin resistance (8, 9, 32). As another mechanism underlying the amelioration of insulin resistance by KRP-101, improvement of serum hypoadiponectinemia may be also considered (33, 44).

It has been reported that PPARα agonists improve adiposity in obese rodents without affecting food intake (15). In the present study, KRP-101 significantly decreased visceral fat weight in dogs fed a high-fat diet under pair-fed conditions, despite the absence of a significant effect on body weight. The effects of KRP-101 under pair-fed conditions suggest increased energy expenditure. Nevertheless, the efficiency with which PPARα agonism may increase energy expenditure in humans remains to be demonstrated experimentally. Therefore, further studies are needed to further clarify the appetite-independent effects of PPARα agonists on body weight and adiposity in dogs fed a high-fat diet.

KRP-101 significantly decreased leptin and increased adiponectin levels in the circulation. Large adipocytes are known to secrete more leptin and less adiponectin in the circulation (11). Therefore, these effects could be associated at least in part with decreased visceral fat weight. We cannot exclude the possibility that circulating levels of adipokines are regulated by other factors, such as insulin and PPAR (3, 10, 22), independent of fat cell size, because the improvement effects reached their values before feeding a high-fat diet.

Our results showed that a specific PPARα agonist, KRP-101, is able to ameliorate the pathogenetic processes, such as fatty liver, hyperglycemia, and adiposity, in dogs fed a high-fat diet. The agonistic activity of KRP-101 in dog PPARα trans-criptional assay was >1,250-fold more potent than that of fenofibric acid, which is a major active metabolite of fenofibrate as a clinically relevant agonist. Whether fibrates are effective for glycemic control for type 2 diabetic patients has been controversial (16, 20, 23). In addition, there is no clear evidence supporting the clinical efficacy of fibrates for obesity and fatty liver (20, 39). Insufficient clinical observations may be explained by the weak and limited effect of fibrates on PPARα (42). Therefore, we would emphasize that it will be important to clinically reevaluate the therapeutic potentials of such treatment using potent and subtype-selective PPARα agonists.

In summary, the results of the present study demonstrate that KRP-101 suppresses insulin resistance and adiposity in a nonrodent disease model. The agonistic activity of KRP-101 is highly potent and subtype-selective compared with classical PPARα agonists, the fibrates. We hypothesized that, in humans, potent and subtype-selective PPARα agonism may offer a therapeutic option for the treatment of insulin resistance, obesity, and dyslipidemia.

REFERENCES


