Beazafibrate regulates the expression and enzyme activity of 11β-hydroxysteroid dehydrogenase type 1 in murine adipose tissue and 3T3-L1 adipocytes

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Nakano S, Inada Y, Masuzaki H, Tanaka T, Yasue S, Ishii T, Arai N, Ebihara K, Hosoda K, Maruyama K, Yamazaki Y, Shibata N, Nakao K. Beazafibrate regulates the expression and enzyme activity of 11β-hydroxysteroid dehydrogenase type 1 in murine adipose tissue and 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab 292:E1213–E1222, 2007. First published December 26, 2006; doi:10.1152/ajpendo.00340.2006.—A clinically employed antihyperlipidemic drug, bezafibrate, has been characterized as a PPAR(α, γ, and δ) pan-agonist in vitro. Recent extended trials have highlighted its anti-diabetic properties in humans. However, the underlying molecular mechanism is not fully elucidated. The present study was designed to explore potential regulatory mechanisms of intracellular glucocorticoid reactivating enzyme, 11β-HSD1 and anti-diabetic hormone, adiponectin by bezafibrate in murine adipose tissue, and cultured adipocytes. Treatment of db/db mice with bezafibrate significantly ameliorated hyperglycemia and insulin resistance, accompanied by a marked reduction of triglyceride and nonesterified fatty acids. Despite equipotent in lipid-lowering effects, another fibrate, fenofibrate, did not show such beneficial effects on glycemic control. Treatment of bezafibrate caused a marked decrease in the mRNA level of 11β-HSD1 preferentially in adipose tissue of db/db mice (−47%, P < 0.05), concomitant with a significant increase in plasma adiponectin level (+37%, P < 0.01). Notably, treatment of bezafibrate caused a marked decrease in the mRNA level (−34%, P < 0.01) and enzyme activity (−32%, P < 0.01) of 11β-HSD1, whereas the treatment substantially augmented the expression (+71%, P < 0.01) and secretion (+27%, P < 0.01) of adiponectin in 3T3-L1 adipocytes. Knockdown of 11β-HSD1 by siRNA confirmed that 11β-HSD1 acts as a distinct oxoreductase in adipocytes and validated the enzyme activity assays in the present study. Effects of bezafibrate on regulation of 11β-HSD1 and adiponectin in murine adipocytes were comparable with those in thiazolidinediones. This is the first demonstration that bezafibrate directly regulates 11β-HSD1 and adiponectin in murine adipocytes, both of which may contribute to metabolically-beneficial effects by bezafibrate.

metabolic syndrome; adiponectin

FAMILY GENES of peroxisome proliferator-activated receptors (PPARs) are profoundly relevant to fuel homeostasis (14, 41). Agonists for PPARα and PPARγ have been widely used for the treatment of dyslipidemia and type 2 diabetes (46). Recent research progress (3) has highlighted the potential usefulness of PPARα/γ dual agonists or PPAR(α, γ, and δ) pan-agonists for metabolic diseases. However, some of these compounds are reported to cause adverse effects, including carcinogenesis, edema, hepatotoxicity, and increase in body weight in rodent experiments (45). Beazafibrate has been widely used for the treatment of dyslipidemia in human clinics (11). Recent in vitro experiments have shown that bezafibrate serves as a pan-agonist for PPARα, γ, and δ (9, 48). Furthermore, recent extended clinical trials (15, 43) have highlighted its anti-diabetic properties. However, the underlying mechanism is not fully clarified. To explore unidentified mechanisms whereby bezafibrate ameliorates metabolic derangement, using genetically obese diabetic K6.Cg-/-Lept/db/db/+Lept/db/db mice, which represent a cluster of detrimental metabolic sequelae such as morbid obesity, diabetes, dyslipidemia, and liver steatosis (39), we examined metabolic response to bezafibrate.

Intracelluar glucocorticoid reactivating enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is abundantly expressed in liver, adipose tissue, skeletal muscle, and central nervous system (38). Targeted overexpression of 11β-HSD1 in adipose tissue in mice results in visceral fat obesity, insulin resistance, and hypertension, suggesting that increased level of 11β-HSD1 plays a critical role in metabolic derangements by providing local glucocorticoid excess within fat cells (29, 30). On the other hand, 11β-HSD1 knockout mice (32), as well as adipose-specific 11β-HSD2 overexpressors, which mimicked adipose-specific 11β-HSD1 knockout mice (18), are protected against the metabolic syndrome under overnutrition. Furthermore, recent studies (36, 47) in rodents suggest that local glucocorticoid excess mediated by 11β-HSD1 in liver or skeletal muscle is also involved in the pathophysiology of the metabolic diseases.

Here, we show that beazafibrate potently decreases the mRNA level of 11β-HSD1 (28) in adipose tissue of db/db mice. We also demonstrate for the first time that bezafibrate inhibits the expression and enzyme activity of 11β-HSD1 in 3T3-L1 adipocytes. Our data show that bezafibrate potently increases the plasma level of antidiabetic adiponectin (26) in mice and augments substantially the expression and secretion of adiponectin in 3T3-L1 adipocytes. To our knowledge, this is the first study to demonstrate that bezafibrate potently regulates 11β-HSD1 and adiponectin in murine fat cells.

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MATERIALS AND METHODS

Materials. Bezafibrate was obtained from Chugai Pharmaceutical (Tokyo, Japan). Fenofibrate and WW-14,643 were purchased from Sigma-Aldrich (St. Louis, MO). Pioglitazone hydrochloride (pioglitazone), fenofibrate, an active metabolite of fenofibrate, and rosiglitazone were synthesized by Kissei Pharmaceutical (Matsumoto, Japan). WW-14,643 and fenofibric acid were used for in vitro experiments.

Animal experiments. Animal experiments in the present study were conducted in accordance with the guidelines for animal experiments of Kissei Pharmaceutical and the Animal Research Committee, Graduate School of Medicine, Kyoto University, and were approved by the Japanese Pharmacological Society. Six-week-old male BKS.Cg-m/db/db (Tokushima, Japan), and AssayMax Corticosterone ELISA kit (AssayMax Corticosterone ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan) with a biochemistry automatic analyzer.

Measurement of metabolic parameters in plasma. Blood samples were collected from tail vein with heparinized hematocrit tube into the collection tube with 50 U aprotinin (Trasylol; Bayer Yakuhin, Osaka, Japan). Fenofibrate and WY-14,643 were purchased from Clea Japan (Tokyo, Japan). Plasma LDL cholesterol and HDL cholesterol were measured by Cholestest LDL and Cholestest N HDL (Daiichi Pure Chemicals, Tokyo, Japan). Plasma insulin, leptin, triglyceride (TG), and nonesterified fatty acids (NEFA) were measured by Insulin ELISA kit, Mouse Leptin ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan), and NEFA C test, respectively; Wako Pure Chemical Industries. Blood samples were collected from tail vein at 0.5, 1, 1.5, and 2 h after insulin injection. The AUC of glucose levels (0–0.5 and 0–1 h) were calculated to assess the status of glucose homeostasis by the linear trapezoidal equation (35).

Intravenous insulin tolerance test. At 2 wk after the treatments were initiated, db/db and lean mice fasted for 12 h were injected 0.8 U/kg body wt insulin (Novo Nordisk Pharma, Tokyo, Japan) via the tail vein. Blood was collected from tail vein at 0.5, 1, 1.5, and 2 h after insulin injection. The AUC of glucose levels (0–0.5 and 0–1 h) were calculated to assess the status of insulin resistance by the equation described above.

TG and total cholesterol contents in liver. At 8 wk after the treatments were initiated, mice were anesthetized with intraperitoneal injection of 20% chloral hydrate (Wako Pure Chemical Industries). Removed liver tissue was quickly weighed, and one part was homogenized and subjected to lipid extraction according to the method of Folch et al. (7). Hepatic TG and cholesterol contents were measured using commercially available kits (TG E-test and cholesterol E-test; Wako Pure Chemical Industries).

Determination of mRNA levels. Total RNA was isolated from liver, mesenteric fat, subcutaneous fat, triceps surae muscle, and 3T3-L1 adipocytes by Isogen (Nippon Gene, Tokyo, Japan). The mRNA level corresponding to a couple of target genes was determined by real-time quantitative RT-PCR using a GeneAmp 5700 Sequence Detection System (Applied Biosystems, Foster City, CA). Primers and double-dye probes for carnitine palmitoyltransferase (CPT) I, acyl-CoA oxidase (ACO), uncoupling protein (UCP3), and adiponectin were designed using Primer Express 2.0 (Applied Biosystems; Table 1). Primers and double-dye probes for 11β-HSD1 and UCP2 were designed as described previously (1, 16). Results were normalized to 18S ribosomal RNA concentration determined by PreDeveloped TaqMan Assay Reagents (Applied Biosystems).

Cell culture and treatment. 3T3-L1 preadipocytes (passages 3–10; American Type Culture Collection, Manassas, VA) were grown and differentiated into adipocytes, as described previously (8). Briefly, differentiation was induced by incubating the cells in DMEM that included 10% FBS with 0.5 mmol/l IBMX, 1 μmol/l dexamethazone, and 10 μg/ml insulin for 2 days, followed by another 2-day incubation in DMEM that included 10% FBS with 10 μg/ml insulin. The cells were further incubated in DMEM with 10% FBS for an additional 4 days to complete the adipocyte conversion. At day 8 following the initiation of differentiation, vehicle (1% DMSO), bezafibrate (1–300 μmol/l), rosiglitazone (3 μmol/l), pioglitazone (10 μmol/l), fenofibric acid (1–300 μmol/l), or WW-14,643 (1–10 μmol/l) were replenished to cultured cells and coincubated from 8 to 48 h at 37°C in 5% CO2.

Table 1. Primers and double-dye probes used for real-time quantitative PCR

<table>
<thead>
<tr>
<th>Name</th>
<th>Oligonucleotide Sequence</th>
<th>Amplicon Size, bp</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACO</td>
<td>Forward: 5’-TGAGACCCGCCCCCCATGAC-3’</td>
<td>72</td>
<td>AF006688</td>
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<tr>
<td></td>
<td>Reverse: 5’-GCGAAGGCCGCCGAAGC-3’</td>
<td>-</td>
<td>AF017175</td>
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<tr>
<td></td>
<td>Probe: FAM5’-TCTGAGACGACTGAATGTGTTCTGCTTCTCCTCC-3’ TAM</td>
<td>-</td>
<td>NM_009464</td>
</tr>
<tr>
<td>CPT I</td>
<td>Forward: 5’-CGTCCCGGGATCATGTTG-3’</td>
<td>87</td>
<td>U37222</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’-AACCTGACTGATGTTTCTC-3’</td>
<td>-</td>
<td>AF006688</td>
</tr>
<tr>
<td></td>
<td>Probe: FAM5’-CGCATGGGCCAGATGTTGCAAGC-3’ TAM</td>
<td>-</td>
<td>AF017175</td>
</tr>
<tr>
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<td>Forward: 5’-CGGCAACACGAGACAGAA-3’</td>
<td>79</td>
<td>AF006688</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’-TGGCGAGGAGAGGGAGA-3’</td>
<td>-</td>
<td>AF017175</td>
</tr>
<tr>
<td></td>
<td>Probe: FAM5’-TCTGCAAGACGATTCTTCTTGAG-3’ TAM</td>
<td>-</td>
<td>AF017175</td>
</tr>
</tbody>
</table>

ACO, acyl-CoA oxidase; CPT I, carnitine palmitoyltransferase I; UCP3, uncoupling protein 3.

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Suppression of 11β-HSD1 expression by RNA interference. 11β-HSD1 expression in 3T3-L1 differentiated adipocytes was suppressed by a small interfering RNA (siRNA) duplex oligonucleotide-targeted 11β-HSD1 mRNA sequence. Individual siRNA duplex was designed using a siRNA Design Support System (TaKaRa Bio, Shiga, Japan). 3T3-L1 differentiated adipocytes (day 8) were transfected with the siRNA duplex (10 nmol/l; sense: 5'-GAAUUGGCAUAUCAUCUGUTT-3' and antisense: 3'-TTCCUUACCUGAUAGUGAGACA-5') using TransIT-TKO Transfection Reagent (Mirus Bio, Madison, WI) for 48 h. Negative Control siRNA (Qiagen, Tokyo, Japan) was used as a nonsilencing control.

Measurement of 11β-HSD1 enzyme activity. Oxoreductase enzyme activity of 11β-HSD1 was assessed according to a previous report (5), with slight modification. Assays for 11β-HSD1 activity were performed by incubating intact cells with corticosteroids with appropriate tritiated tracer. In assays for oxoreductase activity, cells were incubated in serum-free DMEM containing 250 nmol/l cortisone with appropriate tritium-labeled tracer [1,2-3H2]cortisone (50 Ci/mmol; American Radiolabeled Chemicals, St. Louis, MO). After the incubation at 37°C for indicated time, corticosteroids were extracted using ethyl acetate, separated by thin layer chromatography (TLC) in chloroform-methanol (95:5), and quantified by autoradiography. 11β-HSD1 oxoreductase activities were expressed as pmol product ([1,2-3H2]cortisol)−min−1×well−1 after correction for apparent conversion in reactions without cells.

Western blot analysis. 3T3-L1 pre- or differentiated adipocytes were cocultivated with drugs or vehicle for 24 h. Proteins in cultured media (25 ng/lane) were separated by standard Laemmli’s method (23) and subjected to immunoblotting. Western blot analyses were performed using anti-mouse adiponectin antibody (MAB3608; Chemicon International, Temecula, CA).

Statistical analyses. All data were expressed as means ± SE. All statistical analyses were performed using SAS System Version 8.2 (SAS Institute, Cary, NC) and its interlocking movement program. Student’s t-test or Dunnett’s multiple comparison test, followed by univariate repeated-measures ANOVA, taking pretreatment values as covariate. Differences were considered significant at P < 0.05.

RESULTS

Body weight and food consumption. Increment in body weight and food consumption in db/db mice was exaggerated compared with lean mice during the course of the experiment (P < 0.01). Both bezafibrate and fenofibrate tended to increase body weight compared with vehicle-treated db/db mice (P = 0.055 by ANOVA; Table 2).

Liver and mesenteric fat weight. Liver and mesenteric fat weight in vehicle-treated db/db mice was significantly elevated compared with vehicle-treated lean mice (P < 0.01; Table 2). Treatments of both bezafibrate and fenofibrate significantly increased liver weight compared with vehicle-treated db/db mice, whereas increase in liver weight in fenofibrate treatment was exaggerated compared with that in bezafibrate treatment (Table 2). It should be noted that both treatments did not significantly increase mesenteric fat weight (P = 0.63 by ANOVA; Table 2).

Plasma lipid parameters. Plasma levels of TG, NEFA, HDL cholesterol, and LDL cholesterol in vehicle-treated db/db mice at 15 wk of age were significantly higher than those in vehicle-treated lean mice (P < 0.01; Table 2). Both bezafibrate and fenofibrate markedly lowered plasma TG levels to around normal range at 8 wk after the treatments were initiated (Table 2). In addition, both treatments significantly lowered plasma level of NEFA (Table 2). Furthermore, both compounds significantly increased HDL cholesterol level in plasma (Table 2). In contrast, both compounds did not alter LDL cholesterol level in plasma (Table 2).

Hepatic TG and total cholesterol contents. Hepatic TG content in vehicle-treated db/db mice at 15 wk of age was significantly higher than that in vehicle-treated lean mice (P < 0.05 by ANOVA; Table 2). Bezafibrate and fenofibrate markedly lowered plasma TG levels to around normal range at 8 wk after the treatments were initiated (Table 2). It should be noted that both treatments did not significantly increase mesenteric fat weight (P = 0.63 by ANOVA; Table 2).

Table 2. Body weight, food consumption, liver weight, mesenteric fat weight, plasma lipid level, and hepatic lipid content in db/db and vehicle-treated lean mice

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Vehicle</th>
<th>Bezafibrate</th>
<th>Fenofibrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g (15 wk of age)</td>
<td>29±0.7±**</td>
<td>34±1</td>
<td>39±1</td>
<td>38±3</td>
</tr>
<tr>
<td>Average food consumption, g (7–15 wk of age)</td>
<td>5.1±0.2±**</td>
<td>7.9±0.1</td>
<td>7.7±0.1</td>
<td>7.1±0.1</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>1.7±0.1±**</td>
<td>2.3±0.1</td>
<td>3.3±0.1**</td>
<td>3.7±0.4**</td>
</tr>
<tr>
<td>Mesenteric fat weight, mg</td>
<td>264±29±**</td>
<td>598±70</td>
<td>771±91</td>
<td>729±160</td>
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<tr>
<td><strong>Plasma lipid parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>113±10±**</td>
<td>346±35</td>
<td>201±9**</td>
<td>135±15**</td>
</tr>
<tr>
<td>NEFA, mEq/l</td>
<td>0.7±0.1±*</td>
<td>2.1±0.2</td>
<td>1.4±0.2*</td>
<td>1.4±0.2*</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>40±2±**</td>
<td>76±5</td>
<td>96±4**</td>
<td>98±6**</td>
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<td>LDL cholesterol, mg/dl</td>
<td>8±0±**</td>
<td>15±1</td>
<td>14±2</td>
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<tr>
<td><strong>Hepatic lipid contents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride, mg/g liver</td>
<td>4.9±0.8±*</td>
<td>12.2±0.7</td>
<td>10.3±0.4</td>
<td>8.4±0.5*</td>
</tr>
<tr>
<td>Total cholesterol, mg/g liver</td>
<td>1.2±0.2</td>
<td>1.6±0.1</td>
<td>1.1±0.1**</td>
<td>0.9±0.1**</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE (n = 7–8 in each). Values for bezafibrate and fenofibrate are given in mg·kg⁻¹·day⁻¹. NEFA, nonesterified fatty acids. ##P < 0.01 vs. vehicle-treated db/db mice (Student’s t-test). *P < 0.05; **P < 0.01 vs. vehicle-treated db/db mice (1 way ANOVA followed by Dunnett’s multiple comparison test).
Plasma glucose and insulin levels. Plasma glucose levels in vehicle-treated db/db mice at 15 wk of age were significantly higher than those in vehicle-treated lean mice (P < 0.01; Fig. 1A). Bezafibrate significantly reduced plasma glucose levels at 8 wk after the treatments were initiated. In contrast, fenofibrate did not show beneficial effects on plasma glucose levels (Fig. 1A). Plasma insulin levels in vehicle-treated db/db mice at 15 wk of age tended to be higher than those in vehicle-treated lean mice (P = 0.12; Fig. 1B). Both bezafibrate and fenofibrate showed a trend toward reducing plasma insulin levels at 8 wk after the treatments were initiated (P = 0.78 by ANOVA; Fig. 1B).

OGTT and IVITT. To further assess the potential impact of drug administration on glucose tolerance and insulin sensitivity, OGTT and IVITT were performed in db/db mice and lean mice. At 4 wk after the treatments were initiated, bezafibrate, pioglitazone, and rosiglitazone significantly lowered plasma glucose levels compared with vehicle-treated db/db mice, but not fenofibrate (bezafibrate: 117 ± 21 mg/dl, P = 0.0004; fenofibrate: 197 ± 23 mg/dl, P = 0.24; pioglitazone: 143 ± 21 mg/dl, P = 0.006; rosiglitazone: 147 ± 27 mg/dl, P = 0.007 vs. vehicle-treated db/db mice; Fig. 2A). Glucose levels at each time point were significantly elevated in vehicle-treated db/db mice compared with vehicle-treated lean mice (Fig. 2A). AUC0–0.5 h and AUC0–1 h in vehicle-treated db/db mice were significantly higher than those in vehicle-treated lean mice (Table 3). Bezafibrate, pioglitazone, rosiglitazone and fenofibrate significantly reduced AUC0–0.5 h and AUC0–1 h (Table 3). Reduction of AUC0–0.5 h and AUC0–1 h in bezafibrate was exaggerated compared with fenofibrate.

At 2 wk after the treatments were initiated, bezafibrate and rosiglitazone significantly lowered plasma glucose levels compared with vehicle-treated db/db mice, but not pioglitazone and fenofibrate (bezafibrate: 197 ± 22 mg/dl, P = 0.005; fenofibrate: 253 ± 21 mg/dl, P = 0.20; pioglitazone: 258 ± 35 mg/dl, P = 0.31; rosiglitazone: 216 ± 28 mg/dl, P = 0.021 vs. vehicle-treated db/db mice; Fig. 2B). At 0.5 h after the insulin injection in IVITT, bezafibrate, pioglitazone, and rosiglitazone significantly reduced plasma glucose levels (P < 0.05). On the other hand, glucose-lowering effects in fenofibrate-treated mice were marginal (P = 0.29; Fig. 2B). AUC0–0.5 h and AUC0–1 h in vehicle-treated db/db mice were significantly higher than those in vehicle-treated lean mice (Table 3). Bezafibrate, pioglitazone, and rosiglitazone significantly reduced the value of AUC0–0.5 h, and reduction of AUC0–1 h in bezafibrate was equipotent to rosiglitazone (Table 3). In contrast, fenofibrate represented no significant effects on AUCs.

Plasma leptin, adiponectin, and corticosterone levels. Plasma leptin levels in vehicle-treated db/db mice were markedly elevated compared with vehicle-treated lean mice (P < 0.01). Treatments of bezafibrate and fenofibrate did not change plasma leptin levels significantly in db/db mice (P = 0.08 by ANOVA), whereas pioglitazone significantly decreased the levels (Fig. 1C).

Plasma adiponectin levels in vehicle-treated db/db mice were significantly lower than those in vehicle-treated lean mice (P < 0.01; Fig. 1D). Treatments of bezafibrate, pioglitazone, and rosiglitazone caused a significant rise in plasma adiponec-
compared with vehicle-treated
markedly increased the levels for CPT I (Table 4). On the other hand, both bezafibrate and fenofibrate
differences in mRNA level of CPT I, ACO, UCP2, or UCP3
fatty acid
well known (2) that the activation of PPAR
ng/ml; pioglitazone: 132.6
higher than those in vehicle-treated lean mice (Fig. 3). In liver,
Treatments of bezafibrate, fenofibrate, and pioglitazone did not
results of plasma corticosterone levels in db/db mice
(133.7
/ H11006 / bezafibrate: 122.7
/ H9252
muscle, mesenteric fat, and subcutaneous fat, 11
-glucose level in OGTT or IVITT
db/db
vehicle-treated
Lean 68 100 300 300
Vehicle 100 300
Bezafibrate 100 300
Bezafibrate 300 115 15 15
Bezafibrate 300 115 15 15
Bezafibrate 300 152 14 14
Bezafibrate 300 152 14 14
Bezafibrate 300 152 14 14
Bezafibrate 300 152 14 14
Pioglitazone 30 128 11 11
Pioglitazone 30 128 11 11
Rosiglitazone 10 122 15 15
Rosiglitazone 10 122 15 15

Table 3. Effects of bezafibrate, fenofibrate, pioglitazone, rosiglitazone, or vehicle on AUC of plasma glucose level in OGTT or IVITT

Data are presented as means ± SE (n = 8–10 in each). OGTT, oral glucose tolerance test; IVITT, intravenous insulin tolerance test. #/ P < 0.01 vs. vehicle-treated db/db mice (Student’s t-test); * P < 0.05; ** P < 0.01 vs. vehicle-treated db/db mice (1-way ANOVA followed by Dunnett’s multiple comparison test).

Comparison of genese related to fuel metabolism in liver. It is well known (2) that the activation of PPARα induces mRNA expression of a series of genes involved in lipid handling and fatty acid β-oxidation. In liver, there were no significant differences in mRNA level of CPT I, ACO, UCP2, or UCP3 between vehicle-treated db/db and vehicle-treated lean mice (Table 4). On the other hand, both bezafibrate and fenofibrate markedly increased the levels for CPT I (P = 0.30 by ANOVA), ACO, UCP2, and UCP3 (bezafibrate, P = 0.23) compared with vehicle-treated db/db mice (Table 4).

Expression of 11β-HSD1 mRNA. In liver, triceps surae muscle, mesenteric fat, and subcutaneous fat, 11β-HSD1 mRNA levels in vehicle-treated db/db mice were significantly higher than those in vehicle-treated lean mice (Fig. 3). In liver,
bezafibrate, fenofibrate, and pioglitazone significantly decreased the 11β-HSD1 mRNA levels (Fig. 3A). In triceps surae muscle, bezafibrate and fenofibrate also showed a trend toward a decrease in the 11β-HSD1 mRNA levels, but these effects were marginal (Fig. 3A).

Of note, in mesenteric fat, bezafibrate and pioglitazone substantially decreased the 11β-HSD1 expression levels, whereas fenofibrate did not affect the levels (Fig. 3B). In subcutaneous fat, bezafibrate also showed a trend toward lower levels of 11β-HSD1, which were comparable to those in pioglitazone. In contrast, fenofibrate had no effects (Fig. 3B).

mRNA expression levels and enzyme activity of 11β-HSD1 in bezafibrate-treated 3T3-L1 adipocytes. The mRNA levels of 11β-HSD1 in 3T3-L1 differentiated adipocytes were apparently higher than those in vehicle-treated 3T3-L1 preadipocytes. Importantly, bezafibrate, rosiglitazone, and pioglitazone markedly reduced the 11β-HSD1 mRNA levels in 3T3-L1 differentiated adipocytes at 48 h after the treatments. In contrast, fenofibrate and WY-14,643 had no effect on 11β-HSD1 mRNA levels (Fig. 4A).

To further explore the impact on enzyme activity of 11β-HSD1, cells were incubated with tritium-labeled cortisol. Assay for 11β-HSD1 activity clearly showed that 11β-HSD1 acted as distinct oxoreductase in 3T3-L1 differentiated adipocytes (Vehi; Fig. 4B). Bezafibrate, rosiglitazone, and pioglitazone considerably inhibited cortisone-to-cortisol conversion for 48-h treatment (Fig. 4B). Actually, bezafibrate, rosiglitazone, and pioglitazone markedly reduced 11β-HSD1 oxoreductase ac-

Table 4. Hepatic mRNA levels related to fuel metabolism in db/db and vehicle-treated lean mice

Data are presented as means ± SE (n = 7–8 in each). Values for bezafibrate and fenofibrate are given in mg·kg⁻¹·day⁻¹. * P < 0.05; ** P < 0.01 vs. vehicle-treated db/db mice (1-way ANOVA followed by Dunnett’s multiple comparison test).
BEZAFIBRATE REGULATES 11β-HSD1 IN MURINE ADIPOCYTES

Recent extensive clinical trials (43) provided compelling evidence that bezafibrate reduces the incidence of type 2 diabetes in patients with coronary artery diseases. To our knowledge, however, the underlying molecular mechanism of its metabolically beneficial effects has not been fully elucidated. The present study confirmed that repeated administration of bezafibrate significantly ameliorated hyperglycemia and insulin resistance in db/db mice. Nevertheless, antihyperlipidemic effects of fenofibrate were equipotent to bezafibrate (Table 2), and improvement of glucose homeostasis by fenofibrate was marginal compared with bezafibrate. In the present study, 100 or 300 mg·kg⁻¹·day⁻¹ of bezafibrate and 300 mg·kg⁻¹·day⁻¹ of fenofibrate were administered. Because the sensitivity of PPARα agonists in terms of fuel homeostasis in rodents is known to be much lower than in humans, higher doses have been commonly used for rodent experiments (12, 22, 49). Administered doses of compounds were decided mainly on the basis of their plasma TG-lowering effects. In our pilot study, treatment of bezafibrate or fenofibrate (100 mg·kg⁻¹·day⁻¹) in db/db mice for 8 wk did not significantly lower plasma levels of TG. In the next pilot study, treatment of bezafibrate (300 mg·kg⁻¹·day⁻¹) or fenofibrate (300 mg·kg⁻¹·day⁻¹) in db/db mice for 6 wk equipotently lowered plasma TG level (Veh: 204 ± 18 mg/dl; bezafibrate: 95 ± 7, P < 0.01 vs. Veh; fenofibrate: 85 ± 5 mg/dl, P < 0.01 vs. vehicle-treated db/db mice). Notably, treatment of bezafibrate (300 mg·kg⁻¹·day⁻¹) significantly lowered plasma glucose level at 6 wk with an equipotency to rosiglitazone (Veh: 473 ± 24 mg/dl; bezafibrate: 216 ± 40 mg/dl, P < 0.01; fenofibrate: 408 ± 30 mg/dl, P = 0.10; rosiglitazone: 197 ± 23 mg/dl, P < 0.01 vs. vehicle-treated db/db mice). In the present study, we evaluated antidiabetic properties of bezafibrate and fenofibrate with the dose showing equipotency TG-lowering profile. In this context, TG-lowering profile in bezafibrate and fenofibrate in the present study indicates that the doses of drugs used were appropriate for assessing antidiabetic effects. Because we focused our special attention on PPARα agonistic properties of bezafibrate, fenofibrate was employed as a “selective” PPARα agonist. Therefore, we evaluated two doses (100 or 300 mg·kg⁻¹·day⁻¹) of bezafibrate and a single dose (300 mg·kg⁻¹·day⁻¹) of fenofibrate.

Our results raise a couple of possibilities responsible for metabolically beneficial effects of bezafibrate. Decline of adiponectin levels is known (17, 33, 50) to associate with insulin resistance, hepatic fibrosis, and atherosclerosis in humans and rodents. In accordance with previous reports (10), plasma levels of adiponectin in db/db mice were significantly lower than those in vehicle-treated lean littermates (Fig. 1). Notably, bezafibrate significantly increased plasma adiponectin levels in db/db mice, whereas fenofibrate did not provoke such effects. More importantly, we demonstrated for the first time that bezafibrate pronouncedly augmented the expression and secretion of adiponectin in 3T3-L1 differentiated adipocytes (Fig. 5). Activation of PPARγ is known to increase plasma adiponectin levels in humans and rodents (27, 52). First, the present study confirmed that pioglitazone and rosiglitazone increased plasma adiponectin levels in db/db mice (Fig. 1D),...
and rosiglitazone induced adiponectin expression and secretion in 3T3-L1 differentiated adipocytes (Fig. 5). To date, there has been only one report (31) that bezafibrate significantly increased plasma adiponectin levels in obese-diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Moreover, there were some reports (20, 21) showing that fenofibrate increased circulating adiponectin levels in humans. On the other hand, there was only one report (6) that fenofibrate increased mRNA levels of adiponectin in fat depots but did not increase serum levels in OLETF rats. A recent report (44) showed that WY-14,643, a selective PPARα agonist, did not increase serum adiponectin levels in OLETF rats. Collectively, the mechanistic link between PPARα activation and elevation of circulating adiponectin still remains unclear. In the present study, fenofibrate and WY-14,643 did not change adiponectin secretion in 3T3-L1 adipocytes, suggesting that PPARα activation by fenofibrate and WY-14,643 does not exert direct effects on adiponectin secretion. On the other hand, our data support the notion that bezafibrate directly influences adiponectin secretion in 3T3-L1 adipocytes. In contrast, several previous works (20, 21) have suggested that fenofibrate indirectly increased circulating adiponectin, presumably as a reflection of systemic improvement of fuel homeostasis. Our data suggest that bezafibrate-induced increase in plasma adiponectin levels is independent of PPARα activation. Thus, it is likely that a significant increase in adiponectin level induced by bezafibrate may be attributable to its PPARα agonistic activity (48) and thus contribute, at least partly, to the improvement of glucose metabolism in db/db mice. Future investigations to see whether bezafibrate could increase circulating adiponectin level in humans will be of great interest.

A previous report (19) demonstrated that fenofibrate significantly reduced plasma leptin levels in OLETF rats. In the present study, bezafibrate and fenofibrate did not significantly change plasma leptin levels. In contrast, consistent with previous reports (34, 37, 51), pioglitazone decreased plasma leptin...
levels, presumably via PPARγ activation in adipocytes (Fig. 1C). Taken together, a possible mechanistic link between PPARα activation and leptin regulation remains obscure. There were a couple of studies (6, 19) showing that fenofibrate decreased body weight, as well as adipose tissue weight, in OLETF rats. In the present study, both bezafibrate and fenofibrate treatments tended to increase body weight and mesenteric fat weight (Table 2). Because body weight increase was almost parallel to the increment in liver weight (Table 2), it is likely that weight gain in both groups was largely attributable to liver hypertrophy caused by PPARα activation.

Notably, with an equipotency to pioglitazone, bezafibrate markedly lowered the expression level of 11β-HSD1 in mesenteric fat (Fig. 3B). With a sharp contrast, fenofibrate did not change the expression of 11β-HSD1. Importantly, bezafibrate markedly lowered the expression and enzyme activity of 11β-HSD1 in 3T3-L1 differentiated adipocytes, equipotent to rosiglitazone and pioglitazone (Fig. 4). To our knowledge, this is the first demonstration that bezafibrate potently reduces mRNA expression and enzyme activity of 11β-HSD1 in cultured adipocytes. Our data clearly demonstrated that a knockdown of 11β-HSD1 by transfection of siRNA duplex significantly reduced mRNA expression and enzyme activity of 11β-HSD1 in cultured adipocytes. We further demonstrated that bezafibrate, fenofibrate, and pioglitazone significantly decreased 11β-HSD1 mRNA levels in liver (Fig. 3A). These effects may also explain unidentified, metabolically beneficial properties of fibrates (13, 25), because transgenic mice overexpressing 11β-HSD1 exclusively in liver exemplify fatty liver, glucose intolerance, dyslipidemia, and severe hypertension (36). In this context, the potential pathophysiological role of 11β-HSD1 in liver must await further investigation.

Bezafibrate has been characterized as a PPARα, -γ, and -δ pan-agonist in vitro (9, 48). It was reported (24, 40, 42) that GW501516, known as a PPARδ agonist, exerts beneficial effects on insulin resistance. Thus, the activation of PPARδ by bezafibrate may also contribute to its metabolically beneficial effects. Although physiological relevance and an entire picture of the mechanism whereby bezafibrate ameliorates fuel homeostasis in humans must await further investigation, our results presented here provide novel evidence that bezafibrate directly regulates 11β-HSD1 and adiponectin in murine adipocytes. Further investigations on other rodent models and human clinics should validate its accuracy and may open a fresh avenue for therapeutic strategies that might target convergence of multiple risk factors.

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