An Adipose Tissue-Independent Insulin-Sensitizing Action of Telmisartan: a Study in Lipodystrophic Mice

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ABSTRACT

Adipose tissue plays an important role in energy balance and metabolism and is the major target for insulin-sensitizing peroxisome proliferator-activated receptor (PPAR-γ) agonists. The angiotensin II type 1 receptor blocker telmisartan, a partial agonist of PPAR-γ, has been demonstrated to improve insulin sensitivity. However, there is uncertainty about the sites of its action. Here, we demonstrate that treatment with telmisartan (3 mg/kg p.o.) for 7 weeks decreased plasma glucose levels in oral glucose and insulin tolerance tests and the index of the homeostasis model assessment of insulin resistance in A-ZIP/F-1 transgenic mice, an animal model of lipodystrophy. These effects were accompanied by decreases in circulating triglyceride and fatty acid levels. However, this treatment did not affect body weight and plasma adiponectin, leptin, and corticosterone levels. In A-ZIP/F-1 mouse liver the transcripts encoding PPAR-γ and its downstream lipogenic genes were highly up-regulated, consistent with increased hepatic triglyceride content and lipid droplet accumulation. Telmisartan reversed these effects and also down-regulated mRNAs encoding gluconeogenic genes. Thus, the present findings are consistent with a novel mode of insulin-sensitizing action of telmisartan, involving an adipose tissue-independent pathway. Telmisartan-elicited down-regulation of hepatic expression of PPAR-γ-regulated lipogenic genes is associated with amelioration of fatty liver.

Insulin resistance is a key component of the metabolic syndrome and both precedes and predicts the development of type 2 diabetes. Moreover, even in the absence of diabetes, insulin resistance increases the risk of nonalcoholic steatohepatitis and cardiovascular disease (Prasad and Quyyumi, 2004).

Telmisartan is a well established angiotensin II type 1 receptor (AT1) blocker. It has been demonstrated that telmisartan improves insulin sensitivity in rodents that have received high-fat–containing diets (Benson et al., 2004; Fujimoto et al., 2004). In light of these findings, we speculated that the antidiabetic effects of telmisartan might depend on PPAR-γ activity (Engeli et al., 2003). For example, adipocyte-derived leptin regulates food intake and energy expenditure and, in conjunction with the adipokine adiponectin, modulates insulin sensitivity. The peroxisome proliferator-activated receptor (PPAR) γ is a member of the ligand-activated nuclear receptor superfamily and is expressed at high levels in adipose tissues (Evans et al., 1991). In addition to its actions at AT1, telmisartan has been shown recently to facilitate the differentiation of 3T3-L1 preadipocytes and to activate PPAR-γ-responsive genes in adipocytes in vitro (Benson et al., 2004; Fujimoto et al., 2004). In light of these findings, we speculated that the antidiabetic effects of telmisartan might depend on PPAR-γ in adipose tissues.
The A-ZIP/F-1 transgenic mouse is an animal model of lipodystrophy in which a dominant-negative protein that impairs gene activation by leucine zipper transcription factors results in the absence of adipose tissue (Moitra et al., 1998). The mice eat, drink, and urinate copiously, grow heavier than their littermates, and exhibit decreased fecundity (Moitra et al., 1998). A-ZIP/F-1 mice are profoundly insulin-resistant, hyperlipidemic, hepatosteatotic, and hypertensive (Chao et al., 2000; Ebihara et al., 2001; Gavrilova et al., 2003; Takemori et al., 2007). In the present study, we tested the role of adipose tissue in the insulin-sensitizing effect of telmisartan in A-ZIP/F-1 mice.

Materials and Methods

Animals and Treatments. The principles of laboratory animal care were followed in the present study. All procedures were conducted in accordance with institutional guidelines and were approved by the Animal Ethics Committee, Kyoto University, Japan. Mice were housed in a temperature-controlled facility (21 ± 1°C; 55 ± 5% relative humidity) with a 12-h light/dark cycle.

The generation and characterization of the “fatless” A-ZIP/F-1 mice have been reported previously (Ebihara et al., 2001). A-ZIP/F-1 mice were on the FVB/N background, produced by breeding of A-ZIP/F-1 males and wild-type (WT) females. Female A-ZIP/F-1 mice and their WT littermates were used in experiments because females are sterile, possibly as a consequence of leptin deficiency, whereas the males were used for breeding purposes. The animals received a standard diet (CLEA, Tokyo, Japan) and water ad libitum. At 16 to 18 weeks of age, systolic blood pressure (SBP) and body weight were measured. For determination of nonfasting plasma levels of glucose, triglyceride, and nonesterified fatty acids (NEFA) by enzymatic methods, five small droplets of blood were collected carefully by orbital puncture under light ether anesthesia. The A-ZIP/F-1 mice were selected and grouped (n = 7 each group) for experiments based on these parameters; these parameters did not differ between animals in the vehicle- and telmisartan-treated groups. Two experiments were performed. 1) Higher-dosage experiment: telmisartan (3 mg/kg, a generous gift from Boehringer Ingelheim Co., Ltd, Tokyo, Japan, suspended in 5% gum arabic) was administered by oral gavage, once daily (11:00–12:00 AM) for 7 weeks, whereas controls received 5% gum arabic alone. Mice were weighed every 3 to 4 days, and food intake was estimated weekly. SBP was measured at week 1. Eight small droplets of nonfasted and/or fasted (12 h) blood were collected for determination of plasma levels of adiponectin, leptin, corticosterone, glucose, and lipids at week 4. Oral glucose tolerance test (OGTT) and intraperitoneal insulin tolerance test (ITT) were performed at weeks 5 and 6, respectively. Liver was dissected and weighed after animals were killed, and a segment of liver was immediately frozen in liquid nitrogen and stored at −80°C; these parameters did not differ between animals in the vehicle- and telmisartan-treated groups. Tissue triglyceride content was determined as described previously (Oakes et al., 2001). In brief, 100 mg of liver was homogenized and extracted with 2 ml of isopropyl alcohol. After centrifugation, triglyceride content in the supernatant was determined with an enzymatic colorimetric method (Wako).

Gene Expression Analysis. RNA was extracted from the livers of individual mice using TRIzol (Invitrogen, Osaka, Japan). Single-stranded cDNA was synthesized from 1 μg of total RNA by use of the SuperScript III First-Strand Synthesis System (Invitrogen). Quantitative real-time PCR was performed with an AB 7300 Real-Time PCR System using TaqMan (Applied Biosystems, Foster City, CA). The primers and probes (α-Genosys, Hokkaido, Japan) used are shown in Table 1. Mouse mitochondrial subunit 18S rRNA was selected as the endogenous control gene.

PPAR-γ protein was quantified by Western blotting (Lorenzo et al., 2002). Tissue proteins were resolved on 4 to 12% polyacrylamide gels in the presence of sodium dodecyl sulfate, transferred electrophoretically to polyvinylidene difluoride membranes, blocked (in buffer containing 20 mM Tris, pH 7.5, 150 mM NaCl, 5% bovine serum albumin, 0.1% Tween 20), and incubated at 4°C for 18 h with PPAR-γ-specific antibody (1:800; Cell Signaling, Danvers, MA). Detection was performed with peroxidase-conjugated secondary antibody by enhanced chemiluminescence (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Immunoblotting with a monoclonal anti-β-actin antibody (Cell Signaling) was conducted to ensure equal protein loading.

Data Analysis. All results are expressed as means ± S.E.M. Data from more than two experimental groups were analyzed by one-way analysis of variance (ANOVA). The Student-Newman-Keuls test was performed to identify the differences between groups. Data from two experimental groups (Fig. 6) were analyzed by use of the Student’s t-test. P < 0.05 was considered significant.

Results

Higher-Dosage Experiment. In accordance with the previous reports (Moitra et al., 1998; Takemori et al., 2007), A-ZIP/F-1 mice that received a standard diet exhibited higher SBP (Fig. 1A), increased food intake (Fig. 1B), heavier body weights (Fig. 1C), compared with WT mice. It has been reported that there is a 90% reduction in brown adipose tissue and the remaining 10% is inactive in A-ZIP/F-1 mice (Moitra et al., 1998). In contrast to WT mice (Fig. 1D) in the present study, A-ZIP/F-1 mice showed minimal adipose tissue (Fig. 1E). We first tested the effects of telmisartan at higher dosage (3 mg/kg), although this was lower than the dose used in previous studies (5 mg/kg) (Benson et al., 2004; Araki et al., 2006; Sugimoto et al., 2006). Telmisartan treatment lowered SBP to the level observed in WT mice (Fig. 1A), but exerted minimal effects on parameters relating to food...
intake (Fig. 1B), body weight (Fig. 1C), or adipose tissue (Fig. 1F, by gross necropsy) in A-ZIP/F-1 mice.

At week 4, plasma glucose levels in A-ZIP/F-1 mice were significantly increased over those in WT mice under non-fasted conditions, but not after a 12-h fast (Fig. 2A). Fasted plasma insulin levels (Fig. 2B) and the HOMA-IR index (Fig. 2C) were much higher in A-ZIP than in WT mice. Telmisartan treatment markedly decreased plasma insulin concentrations (Fig. 2B) and the HOMA-IR index (Fig. 2C) in A-ZIP/F-1 mice, but was without effect on plasma glucose concentrations (Fig. 2A).

In further studies the responses of plasma glucose to challenge with exogenous glucose and insulin were assessed. Plasma glucose concentrations and glucose AUC did not differ significantly between A-ZIP/F-1 and WT mice either before or after OGTTS were conducted (2 g/kg) at week 5 (Fig. 2, D and E). Telmisartan treatment prevented the increases in plasma glucose concentrations at 20 min and in glucose AUC

### Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer 5' to 3' sequences</th>
<th>Probe 5' to 3' sequences</th>
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<tr>
<td>18S</td>
<td>CGCGCAATTACCCACTCCCGA</td>
<td>f CCGCTACACATCCAGGGA</td>
</tr>
<tr>
<td>PPAR-γ</td>
<td>CTTCCATCAAGAGGATCCACAGAGC</td>
<td>r CCAATTACAGGGCTCGAAA</td>
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<tr>
<td>PEPCK</td>
<td>CAACGTTGCTGGCTCTCACTGACC</td>
<td>f AGAGCTATGGCCCTTGC</td>
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<tr>
<td>G6P</td>
<td>CTCTATGGGAAACCTGCGCAG</td>
<td>r ATGTCAGGAGATGGCTG</td>
</tr>
<tr>
<td>SCD1</td>
<td>CCACCGACCAATGCTCCCTCTCT</td>
<td>f TGTCATCCGCAAGTGCTAGGA</td>
</tr>
<tr>
<td>SREBP1c</td>
<td>CAGCTCATCAACAAACCAAGCAGTGACTTC</td>
<td>f CTTCGATTCGCAACATCT</td>
</tr>
<tr>
<td>FAS</td>
<td>AAACCACTCCTGGGCTCACTTCT</td>
<td>r GCCACCAAGACTTTGCTG</td>
</tr>
<tr>
<td>ACC1</td>
<td>CTCAACCTGGATGTCCTTGTGCCAGC</td>
<td>r CCCGACCAAGACTTTGCTG</td>
</tr>
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* Sequences: 5' to 3'. Forward primers are designated by f and reverse primers by r.
in OGTT. In contrast, the decrease in plasma glucose concentrations elicited by insulin was minimal in A-ZIP/F-1 mice compared with WT control (Fig. 2F), but was more pronounced when telmisartan was coadministered. Consistent with these findings, the glucose AUC after insulin was higher in A-ZIP/F-1 than in WT mice (Fig. 2G), and was decreased by telmisartan treatment.

Plasma adiponectin and leptin levels were extremely low in A-ZIP/F-1 mice and were unaffected by telmisartan treatment (Fig. 3, A and B). In contrast, plasma corticosterone in A-ZIP/F-1 mice was slightly, but not significantly, increased over WT control (Fig. 3C); telmisartan treatment did not affect plasma corticosterone levels in A-ZIP/F-1 mice. The generalized lipodystrophy also led to redistribution of lipids. Nonfasted plasma NEFA levels by 42% and 28%, respectively. Telmisartan for 4 weeks decreased plasma triglyceride and compared with WT mice (Fig. 3, D and E). Treatment with levels of triglyceride and NEFA were elevated in A-ZIP/F-1 mice dystrophy also led to redistribution of lipids. Nonfasted plasma corticosterone in A-ZIP/F-1 mice. The generalized lipo- compared with control animals (Fig. 1D) was partially reversed by telmisartan (Fig. 1F). Indeed, the increase in hepatic triglycerides in A-ZIP/F-1 mice to 7- to 12-fold of WT control was substantially ameliorated by telmisartan (Fig. 4, B and C). In accord with these findings, the extensive fatty infiltration in A-ZIP/F-1 livers (Fig. 4E) compared with control (Fig. 4D), was also reversed in part by telmisartan (Fig. 4F).

Expression of the mRNAs encoding the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6P) was similar in liver of A-ZIP/ F-1 and control mice; both mRNAs were decreased by treatment with telmisartan by 62% and 61%, respectively (Fig. 5, A and B). In livers of A-ZIP/F-1 mice PPAR-γ mRNA expression was increased relative to WT control, which was substantially resolved by telmisartan treatment (Fig. 5C). In accord with these findings, PPAR-γ2 immunoreactive protein was up-regulated in A-ZIP/F-1 mouse liver and normalized by telmisartan treatment, but PPAR-γ1 protein expression was very low and unaltered (Fig. 5D). Hepatic stearoyl-CoA desaturase (SCD) 1, fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) 1 mRNA levels in the A-ZIP/F-1 mice were markedly increased over those in WT mice (Fig. 5, E–G), but the slight increase in sterol regulatory element-binding protein (SREBP) 1c mRNA did not attain statistical significance (Fig. 5H). Consistent with the effect of telmisartan on PPAR-γ gene expression, treatment of A-ZIP/F-1 mice with the AT1 blocker markedly suppressed hepatic mRNAs corresponding to the PPAR-γ-regulated downstream genes SCD1, FAS, ACC1, and SREBP1c.

Lower-Dosage Experiment. We also tested the effects of telmisartan at lower dosage. Treatment with 1 mg/kg telmisartan significantly decreased SBP in A-ZIP/F-1 mice (Fig. 6A). However, this treatment did not affect plasma glucose

**Fig. 2.** Nonfasted and fasted (12 h) plasma glucose (A and B) levels, the index of the HOMA-IR (C) at week 4, and plasma glucose response to exogenous glucose (D and E) and insulin (F and G) challenge in female WT and A-ZIP/F-1 mice. OGTT (glucose: 2 g/kg p.o.) and insulin tolerance test (ITT, insulin: 0.75 IU/kg i.p.) were performed after fasting (12 h) at week 5 and 6, respectively. All values are means ± S.E.M. (n = 7). *, P < 0.05 compared with A-ZIP/F-1 control (Tel 0) (ANOVA). Tel 3, telmisartan 3 mg/kg.
Fig. 3. Nonfasted plasma adiponectin (A), leptin (B), corticosterone (C), triglyceride (D), and NEFA (E) levels in female WT and A-ZIP/F-1 mice (week 7). All values are means ± S.E.M. (n = 7). Versus A-ZIP/F-1 control (Tel 0), * P < 0.05 (ANOVA). Tel 3, telmisartan 3 mg/kg.

Fig. 4. Liver weight (A), liver triglyceride (B), and total liver triglyceride (C) contents, and liver histology in female WT and A-ZIP/F-1 mice (week 7). All values are means ± S.E.M. (n = 7). Versus A-ZIP/F-1 control (Tel 0), * P < 0.05 compared with A-ZIP/F-1 control (Tel 0) (ANOVA). Representative liver pathological changes (hematoxylin and eosin staining, ×200) (D and F). Tel 3, telmisartan 3 mg/kg.
Discussion

PPAR-γ is expressed predominantly in adipose tissue, which facilitates lipid uptake and storage in that tissue and alters the release of leptin and adiponectin, which modulates insulin sensitivity. Adipose PPAR-γ is the molecular target for the insulin-sensitizing thiazolidinediones (Evans et al., 2004). The principal findings to emerge from the present study were that telmisartan treatment (3 mg/kg) enhanced insulin sensitivity and improved the abnormalities of lipid metabolism observed in A-ZIP/F-1 mice. Thus, the present findings indicate that telmisartan has additional beneficial effects that are distinct from adipose tissues. It seems that the improvement in insulin sensitivity in adipose tissue-deficient mice affected by telmisartan is independent of PPAR-γ agonism.

Although the mechanisms underlying insulin resistance in patients with lipodystrophies are unclear, accumulation of triglycerides in liver and skeletal muscle is probably important (Garg, 2004). Intracellular accumulation of fatty acids and triglycerides in nonadipose tissues has been implicated in insulin resistance (Shimabukuro et al., 1997). It has been demonstrated that a decrease in hepatic triglyceride pools leads to improved insulin sensitivity (Neschen et al., 2005; Savage et al., 2006). On the other hand, an increase in blood lipid levels, especially NEFA, modulates the action of insulin. A high plasma NEFA concentration is a risk factor for deterioration of glucose tolerance that is independent of the other parameters relating to insulin resistance or insulin secretion (Charles et al., 1997). Chronically elevated plasma NEFA concentrations stimulate gluconeogenesis, exacerbate hepatic/muscle insulin resistance, and impair insulin secretion in genetically predisposed individuals (Boden 1997; Bergman and Ader, 2000). Decreased availability of precursor substrates, including free fatty acids and glucose, also diminishes hepatic synthesis and export of esterified lipids (Ran et al., 2004). In the present study, amelioration of insulin resistance in the lipodystrophic A-ZIP/F-1 mice by telmisartan treatment was accompanied by a decrease in hepatic triglyceride accumulation and circulating lipid concentrations.

PPAR-γ is expressed normally at low levels in liver, but is strongly up-regulated in the liver of A-ZIP/F-1 mice and seems to contribute to the development of hepatic steatosis.
Moreover, ablation of hepatic PPAR-γ levels, hepatic steatosis was exacerbated (Chao et al., 2000). Treatment of A-ZIP/F-1 mice with rosiglitazone, a thiazolidinedione, lowered circulating lipid levels, hepatic steatosis was exacerbated (Chao et al., 2000). Moreover, ablation of hepatic PPAR-γ decreased hepatic steatosis in A-ZIP/F-1 mice and promoted hyperlipidemia (Gavrilova et al., 2003). Thus, steatosis seems to result from increased triglyceride formation and uptake by the liver in a PPAR-γ-regulated fashion (Gavrilova et al., 2003). In the present study, telmisartan reversed the increase in hepatic PPAR-γ expression in A-ZIP/F-1 mouse liver and also normalized the expression of several PPAR-γ-responsive genes that participate in fatty acid and triglyceride synthesis. Whereas thiazolidinediones decreased serum triglycerides and free fatty acids, glucose and insulin regulation was not restored (Chao et al., 2000). Thus, the present findings clearly distinguish the effects of telmisartan from PPAR-γ agonist thiazolidinediones. The precise underlying molecular mechanism by which telmisartan modulates hepatic PPAR-γ activity should now be explored further.

Energy homeostasis is regulated at the level of food intake, overall activity, sympathetic tone, energy expenditure, and insulin sensitivity (Flier, 1997). Serum leptin concentrations in A-ZIP/F-1 mice are ~5% of those in WT mice (Moitra et al., 1998). The adipokine adiponectin acts in concert with leptin to enhance insulin sensitivity and regulate glucose metabolism (Berg and Scherer, 2005). Leptin is secreted by white and brown adipose tissue in proportion to tissue mass (Moitra et al., 1998). Leptin acts through the hypothalamic-pituitary-adrenal axis to regulate insulin sensitivity indirectly (Cusin et al., 1998; Liu et al., 1998). Leptin-deficient A-ZIP/F-1 mouse. In the present study, telmisartan did not affect plasma adiponectin and leptin levels, which indicates that the primary defect due to adipocyte deletion was not overcome.

Although disturbance of the regulatory actions of adipose tissue-derived mediators is important, adrenocortical dysregulation may contribute to insulin resistance (Roberge et al., 2007). Thus, glucocorticoids regulate food intake and metabolism that influence energy utilization (Haluzik et al., 2002) and also stimulate gluconeogenesis via PEPCCK and G6P (Pilkis and Granner, 1992). Leptin deficiency may contribute to hypercorticosteronemia in A-ZIP/F-1 mice (Haluzik et al., 2002). Indeed, combined leptin infusion and adrenalectomy decreased plasma corticosterone levels and improved diabetes in A-ZIP/F-1 mice (Haluzik et al., 2002). In the present study, the small increase in plasma corticosterone...
levels in A-ZIP/F-1 mice was not statistically significant and was minimally affected by telmisartan. Thus it is unlikely that telmisartan improves insulin resistance by modulating adrenal corticosterone production in A-ZIP/F-1 mice.

Telmisartan at lower dosage (1 mg/kg) significantly decreased SBP in A-ZIP mice, which is attributed to AT1 inhibition. However, it did not affect insulin resistance, hyperlipidemia, or fatty liver, and did not decrease the expression of hepatic PPAR-γ-regulated lipogenic genes. These results are distinct from those made after treatment with telmisartan at 3 mg/kg (see above) or rosiglitazone (Chao et al., 2004). Telmisartan at 3 mg/kg (see above) or rosiglitazone (Chao et al., 2004) down-regulates hepatic expression of PPAR-γ and its associated complications, including diabetes, hypertrectangicemia, diabetic nephropathy and, diabetes, steatosis, and recurrence acute pancreatitis caused by prolonged hypertrectangicemia (Garg, 2004; Agarwal and Garg, 2006). Morbidity and mortality in patients with lipodystrophies are frequently manifested as diabetes mellitus, diabetic cirrhosis as a result of steatosis, atherosclerosis, and recurrent acute pancreatitis caused by prolonged hypertrectangicemia (Garg, 2004; Agarwal and Garg, 2006). Current treatments are restricted to managing the metabolic abnormalities of insulin resistance, diabetes, dyslipidemia, and hypertension; to date, no specific therapies have emerged. The present study has identified an adipose-tissue-independent insulin-sensitizing effect of telmisartan. Amelioration of hepatic steatosis was associated with down-regulation of hepatic expression of PPAR-γ-mediated genes responsible for lipid synthesis. Therefore, the present finding that telmisartan improves insulin resistance and its associated complications in A-ZIP/F-1 mice may constitute important information for the development of new strategies to manage patients with lipodystrophy.

Taken together, the present findings are consistent with a novel mode of insulin-sensitizing action of telmisartan, involving an adipose tissue-independent pathway. The precise underlying molecular mechanism by which telmisartan modulates hepatic PPAR-γ activity should now be explored further.

Acknowledgments

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References


