Angiotensin II type 1 receptor blocker ameliorates overproduction and accumulation of triglyceride in the liver of Zucker fatty rats

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Ran, Jianmin, Tsutomu Hirano, and Mitsuru Adachi. Angiotensin II type 1 receptor blocker ameliorates overproduction and accumulation of triglyceride in the liver of Zucker fatty rats. Am J Physiol Endocrinol Metab 287: E227–E232, 2004. First published April 13, 2004; 10.1152/ajpendo.00090.2004.—The effects of angiotensin II type 1 receptor blocker (ARB) on triglyceride (TG) metabolism associated with insulin resistance were explored in Zucker fatty (ZF) rats. Olmesartan medoxomil, a newly developed ARB, was given as a 0.01% drinking solution ad libitum to ZF and Zucker lean (ZL) rats for 4 wk. Olmesartan lowered blood pressure in both strains to the same extent. ZF rats had a markedly low insulin sensitivity index (SI) and glucose effectiveness (SG), together with significantly increased glucose levels. Olmesartan treatment substantially elevated both SI and SG. The ZF rats were hyperlipidemic, with plasma TG levels sixfold higher than those of the ZL rats. Olmesartan remarkably decreased the plasma free fatty acid level in the ZF rats, but it did not exert a significant effect on the plasma TG level. The TG secretion rate assessed by the Triton WR-1339 technique was almost six times higher in the ZF than in the ZL rats, and olmesartan treatment suppressed this TG overproduction by one-half. The TG content in the liver was ten times higher in the ZF than in the ZL rats, and olmesartan halved this high hepatic TG content without affecting the cholesterol content. The fatty liver developed in the ZF rats was ameliorated by olmesartan treatment. Olmesartan treatment had no significant effects on TG metabolism or insulin sensitivity in the ZL rats. Taken in sum, ARB improves the overproduction and accumulation of TG in the liver associated with insulin resistance, and it does so through mechanisms independent of its hypotensive action.

Angiotensin II, the major hormone of the renin-angiotensin system, plays an important role in the pathogenesis of hypertension and atherosclerosis (11). Several lines of evidence have suggested that angiotensin II impairs insulin sensitivity (23, 26), and insulin resistance promotes the development of hypertension by upregulating the number and activity of angiotensin II receptors (22). In several other studies, insulin resistance has been improved in response to treatment with angiotensin I-converting enzyme inhibitors (ACEIs) (10, 13). Angiotensin II receptor blocker (ARB) and ACEIs have both been recommended as first lines of anti-hypertensive treatment in patients with diabetes, but it remains uncertain whether insulin action responds as favorably to the former as it does to the latter.

Hypertriglyceridemia is a major component of the insulin-resistant syndrome (27) and often accompanies type 2 diabetes. Insulin resistance/hyperinsulinemia stimulates hepatic production of very-low-density lipoprotein (VLDL), a major carrier protein of endogenously produced TG (17). Several studies have examined the effects of ARB on plasma triglyceride levels (1, 21), but most have failed to find any favorable effect on hypertriglyceridemia. If the ARBs improve insulin resistance, they might have the power to suppress the TG overproduction associated with insulin resistance. Recent studies on nonalcoholic fatty liver have proposed that the condition is related to insulin resistance (28). It remains largely unknown whether ARB exerts favorable effects on lipid metabolism and fatty liver associated with insulin resistance. To address these issues, we investigated the effects of ARB on TG production and hepatic TG content in the Zucker fatty (ZF) rat, an animal model of insulin resistance with glucose intolerance, hypertriglyceridemia, hypertension, and fatty liver (8).

MATERIALS AND METHODS

Animals. Eight-week-old male ZF rats and Zucker lean (ZL) rats (Charles River Japan, Tokyo, Japan) were fed with standard stock diet containing 60% vegetable starch, 5% fat, and 24% protein. Each group was given drinking water for 4 wk with or without 0.01% olmesartan medoxomil (CS-866; Sankyo, Tokyo, Japan), a specific angiotensin II type 1 receptor antagonist (14). All rats were kept in individual cages on a rotating 12:12-h light-dark cycle with free access to food and water. On the experiment day, food was removed at 9:00 AM, and the drinking water was left available until the start of the experiment at 2:00 PM. All procedures were approved by the Institutional Animal Care and Use Committee of Showa University according to the National Research Council’s Guide for the Care and Use of Laboratory Animals.

Blood pressure and heart rate measurements. Systolic (SBP) and diastolic blood pressures (DBP) and heart rate (HR) were recorded in completely conscious rats with an indirect tail-cuff device (Natsume Seisakusho, Tokyo, Japan). Rats were preheated on a 37°C plate for 22, 30, 40, 60, 90, and 120 min. Plasma was separated by centrifugation at 4°C and stored at −80°C for the subsequent measurement of lipid concentrations 2 days before the FS-IVGTT procedure. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium, a catheter filled with heparin was inserted into a femoral vein 1 h before the experiment, a 50% glucose solution was administered as a bolus injection at a dose of 0.5 g/kg in a basal state, and blood samples (0.1 ml each) were taken at −5, 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 30, 40, 60, 90, and 120 min. Plasma was separated by centrifugation at 4°C and stored at −20°C for the subsequent measurement of glucose and insulin. In the analyses of the FS-IVGTT data, the areas under the curves of glucose (AUCG) and insulin (AUCI) were calculated by the intrapile wage method. Insulin sensitivity index (SI)
and glucose effectiveness (SG), a pair of parameters representing insulin-dependent and glucose-dependent glucose disposal abilities in vivo, respectively, were mathematically evaluated according to Bergman’s minimal-model technique (2). The Marquardt-Levenberg method is used for nonlinear least square estimation of the parameters, and the step size for integration is 1 min. The plasma glucose disappearance rate constant ($K_g$) was calculated as the slope of the least square regression line relating the natural logarithm of the glucose concentrations between 4 and 16 min (20).

TG secretion rate. The TG secretion rate (TGSR) was determined by the Triton WR-1339 technique (3, 30), as described in detail earlier (31). The animals were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital sodium, Triton WR-1339 was intravenously injected at a dose of 600 mg/kg, and blood samples were taken before and 60 and 90 min after the injections. Triton injection at the preliminary studies, we confirmed that acute injections of Triton WR-1339 or glucose had no effect on the lipid content in the liver.

Measurement of lipid content in the liver. The liver was removed after the final sampling in the Triton study or the FS-IVGTT. In preliminary studies, we confirmed that acute injections of Triton WR-1339 or glucose had no effect on the lipid content in the liver. The liver was infused with a large amount of saline via the portal vein to wash out the blood constituent. It was dissected and immediately placed in liquid nitrogen. Total lipids were extracted according to the Bligh and Dyer method (4). Briefly, 1.25 g of tissues were homogenized with 3.75 ml of chloroform-methanol (1:2 by volume) using a hand-held polytron (PT10/35, Lucerne, Switzerland), vigorously vortexed for 15 min, mixed with 1.25 ml of chloroform and then with 1.25 ml of water, and centrifuged briefly at 3,000 rpm to separate the phases. Next, the lower phase was transferred to another tube, and the residue was mixed with 1.88 ml of chloroform for the second-step vortex and centrifugation, and the lower phase obtained by the centrifugation was mixed with the first chloroform phase in the same tube. After evaporation with nitrogen gas at 55°C, the lipid extract was dissolved in 2 ml of 2-propanol. TG and cholesterol were assayed by the enzymatic quantitative method described in Biochemical assays. Liver tissue sections of 0.02 mm thickness were incubated for 24 h with 4% paraformaldehyde, 0.2% picric acid, and 0.5% glutaraldehyde in 0.2 mol/l phosphate buffer (pH 7.4) at 4°C. After being washed for 7 days with 15% sucrose at 4°C, the sections were incubated for 1 h with Sudan IV dye bath to stain the TG.

Biochemical assays. Plasma levels of glucose, total cholesterol (TC), TG, nonesterified fatty acid (NEFA), and high-density lipoprotein cholesterol (HDL-C) were measured in duplicate spectrophotometers (Hitachi U-2000) with standard commercial kits (Wako Pure Chemical Industries, Osaka, Japan). Plasma insulin concentration was determined using a specific insulin ELISA kit for rats (Morinaga, Yokohama, Japan).

and glucose effectiveness (SG), a pair of parameters representing insulin-dependent and glucose-dependent glucose disposal abilities in vivo, respectively, were mathematically evaluated according to Bergman’s minimal-model technique (2). The Marquardt-Levenberg method is used for nonlinear least square estimation of the parameters, and the step size for integration is 1 min. The plasma glucose disappearance rate constant ($K_g$) was calculated as the slope of the least square regression line relating the natural logarithm of the glucose concentrations between 4 and 16 min (20).
**RESULTS**

The ZF rats were heavier than the ZL rats at the initial study and remained heavier during the experimental process. Daily food intake was significantly higher in the ZF rats, and daily food intake was significantly higher in the ZL rats, whereas DBP was comparable between the two groups. The HR was higher in the ZL rats. Treatment with olmesartan lowered both SBP and DBP by similarly large amounts in the two groups without olmesartan treatment. The ZF rats were heavier than the ZL rats at the initial study and remained heavier during the experimental process. Daily food intake was significantly higher in the ZF rats, whereas DBP was comparable between the two groups.

**Statistical analysis.** Data are expressed as means ± SD. One-way ANOVA was used to evaluate differences of means between groups. Correlation between two parameters was analyzed by Pearson’s simple correlation analysis. P < 0.05 was accepted as significant.

**Plasma glucose and insulin levels at baseline and during the FS-IVGTT.** Compared with ZL, ZF rats had markedly higher basal plasma glucose and insulin levels, and they also had higher AUCG and AUCI after intravenous glucose challenge. Figure 1 depicts the changes in plasma glucose and insulin levels at baseline and during the FS-IVGTT. SI, SG, and Kg values determined by Bergman’s minimal model were all substantially reduced in the ZF rats. All of these alterations indicated severe insulin resistance and impaired glucose-dependent glucose disposal. Treatment with olmesartan significantly decreased plasma glucose and insulin levels both in the basal state and during the course of FS-IVGTT. In contrast, olmesartan treatment left the glucose and insulin levels unchanged in ZL animals both in the basal state and during the FS-IVGTT.

**Plasma lipid levels and TGSR.** The ZF rats showed severe hyperlipidemia with markedly higher plasma levels of TG, TC, HDL-C, and NEFA. The non-HDL-C, calculated as TC minus HDL-C, was also significantly higher in this group (Table 3). Olmesartan treatment markedly decreased plasma NEFA levels in the ZF rats. It also decreased the plasma TG levels, but not to a significant degree (P = 0.01). TC, HDL-C, and non-HDL-C all remained unchanged in the ZF rats after olmesartan treatment. In the ZL rats, olmesartan treatment had no significant effects on those lipid parameters. The TGSR for the ZF rats was nearly six times higher than that for the ZL rats (0.56 ± 0.08 vs. 0.10 ± 0.01 mg·min⁻¹·100 g body wt⁻¹, respectively, P < 0.0001; Fig. 2), which was in good agreement with six-times-increased plasma TG levels. Treatment with olmesartan halved the high TGSR in the ZF rats (0.30 ± 0.07 mg·min⁻¹·100 g body wt⁻¹), Olmesartan treatment exerted no significant effect on TGSR in the ZL animals (Fig. 2).

**Lipid content in the liver.** As to be expected from the high plasma TG concentration and TGSR, the TG content in the liver was 10-times higher in the ZF rats than in the ZL rats (22.74 ± 4.27 vs. 2.25 ± 0.58 mg/g tissue). The liver cholesterol content was also increased in the ZF rats (1.52 ± 0.29 vs. 1.18 ± 0.07 mg/g), but not to a significant degree. Treatment with olmesartan halved the high hepatic TG content in the ZF rats (12.86 ± 1.74 mg/g after treatment), whereas no such reductive effect was observed in the ZL group. Olmesartan left

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**Table 2. FS-IVGTT parameters assessed by Bergman’s minimal model (Ref. 2)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ZF Rats</th>
<th>ZL Rats</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>Olmesartan (−)</td>
<td>Olmesartan (+)</td>
</tr>
<tr>
<td>207.98±47.66*</td>
<td>155.73±41.86†</td>
<td>168.63±11.03</td>
</tr>
<tr>
<td>Plasma insulin, μU/ml</td>
<td>389.72±44.23*</td>
<td>283.41±48.14†</td>
</tr>
<tr>
<td>AUCG, μg·dl⁻¹·min⁻¹·10⁻⁴</td>
<td>4.17±0.54*</td>
<td>2.69±0.41†</td>
</tr>
<tr>
<td>AUCI, μU·ml⁻¹·min⁻¹·10⁻⁴</td>
<td>5.02±0.44*</td>
<td>3.64±0.27†</td>
</tr>
<tr>
<td>SI, μU·ml⁻¹·min⁻¹·10⁻⁴</td>
<td>0.31±0.22*</td>
<td>1.15±0.17†</td>
</tr>
<tr>
<td>SG, min⁻¹·10⁻²</td>
<td>1.35±0.51*</td>
<td>3.10±1.12†</td>
</tr>
<tr>
<td>Kg, %/min</td>
<td>2.55±0.34*</td>
<td>2.60±0.11</td>
</tr>
</tbody>
</table>

**Values are means ± SD; n, no. of rats/group.** AUCG, area under curve of glucose; AUCI, area under curve of insulin; SI, insulin sensitivity index; SG, glucose effectiveness; Kg, plasma glucose disappearance constant; *P < 0.01 vs. ZF rats without olmesartan; †P < 0.01 vs. ZF rats without olmesartan; ‡P < 0.05 vs. ZL rats without olmesartan; §P < 0.05 vs. ZF rats without olmesartan.

**Table 3. Plasma lipid levels in ZF and ZL rats with and without olmesartan treatment**

<table>
<thead>
<tr>
<th>Lipid</th>
<th>ZF Rats</th>
<th>ZL Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>Olmesartan (−)</td>
<td>Olmesartan (+)</td>
</tr>
<tr>
<td>428.41±60.24*</td>
<td>396.61±55.71</td>
<td>71.59±20.2</td>
</tr>
<tr>
<td>TC, mg/dl</td>
<td>197.64±46.59*</td>
<td>205.89±45.24</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>106.51±14.23*</td>
<td>96.44±18.51</td>
</tr>
<tr>
<td>Non-HDL-C, mg/dl</td>
<td>100.13±43.01*</td>
<td>103.45±45.57</td>
</tr>
<tr>
<td>NEFA, meq/l</td>
<td>2.70±0.69*</td>
<td>1.98±0.43†</td>
</tr>
</tbody>
</table>

**Values are means ± SD; n, no. of rats/group.** TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; NEFA, nonesterified fatty acid. Non-HDL-C was calculated as TC minus HDL-C. *P < 0.01 vs. ZL rats without olmesartan; †P < 0.01 vs. ZF rats without olmesartan.

**Fig. 2. Triglyceride secretion rate (TGSR) determined by Triton WR-1339 technique in ZF and ZL rats with (solid bars) and without (gray bars) olmesartan. **P < 0.01 vs. ZF rats without olmesartan group.
The liver cholesterol content was unchanged in both groups (Fig. 3). The liver TG content was significantly correlated with the TGSR (r = 0.87, P < 0.01). Histological staining of the livers from ZF rats showed severe lipid accumulation, and treatment with olmesartan markedly reduced lipid droplets in hepatic cells (Fig. 4).

**DISCUSSION**

Recent studies have suggested that angiotensin II, a potential vascular constrictor and a key factor for hypertension, impairs insulin action and causes glucose intolerance (23, 26). If so, the inhibition of angiotensin II can be expected to improve insulin sensitivity. As it turns out, there are some experimental and clinical studies that have already demonstrated that ARBs improve insulin sensitivity (8, 24). The results remain controversial, however, as other studies have failed to find ARB-induced improvements of insulin resistance or glucose intolerance (16, 19). In the present study, we observed improvements in both insulin-dependent and insulin-independent glucose disposal in rats treated with a newly developed ARB, olmesartan. Olmesartan also restored the hyperglycemia in pre- and post-loading of glucose in ZF rats while leaving insulin and glucose metabolism unchanged in control ZL rats. Given that olmesartan significantly decreased blood pressure to the same extent in both ZF and ZL rats, the improvement of insulin sensitivity in ZF rats may not be associated with the hypotensive action. Working on this assumption, we hypothesized that ARB stimulates insulin sensitivity only in an insulin-resistant state. If so, it might explain why the favorable effect of ARB on insulin sensitivity has not been constantly observed. The mechanisms for the amelioration of insulin resistance and glucose intolerance by ARB treatment are largely unknown. We speculated that the inhibited action of angiotensin II dilates the vessels and increases the blood flow in the skeletal muscle. The dilatation of the vessel could promote the activation and translocation of the glucose transporter at the cellular level (9), and the increased blood flow could propel glucose diffusion from the vessels and capillaries to insulin-independent tissues (33). We found that the ARB treatment significantly increased both insulin-dependent (SI) and glucose-dependent glucose disposal (SG). The former increase may imply an enhancement of insulin-mediated glucose transport, and the latter may mainly reflect hemodynamic changes for facilitating passive glucose transport in the normoglycemic state.

ZF rats had sixfold higher plasma TG than controls, which was to be expected from the sixfold elevation in their rate of liver cholesterol content unchanged in both groups (Fig. 3). The liver TG content was significantly correlated with the TGSR (r = 0.87, P < 0.01). Histological staining of the livers from ZF rats showed severe lipid accumulation, and treatment with olmesartan markedly reduced lipid droplets in hepatic cells (Fig. 4).

Fig. 3. Liver triglyceride (TG) content (A) and total cholesterol (TC, B) content in ZF and ZL rats with (solid bars) and without (gray bars) olmesartan treatment. Total lipid in the liver was extracted according to the Bligh and Dyer method. **P < 0.01 vs. ZF rats without olmesartan group.

Fig. 4. Representative histological staining of livers from ZL rats, ZF rats, and ZF rats treated with olmesartan. Samples were stained with Sudan IV. ZF rats showed marked lipid accumulation, and treatment with olmesartan significantly reduced lipid droplets in hepatic cells.
TG production. Peinado-Onsurbe et al. (25) found no decrease in the activity of lipoprotein lipase, a key enzyme for TG hydrolysis, in ZF rats, suggesting that the severe hypertriglyceridermia in ZF rats is solely due to TG overproduction. Because TG production is stimulated in an insulin-resistant state (29), the insulin resistance in ZF rats can be reasonably assumed to cause TG overproduction. ZF rats exhibited a significant elevation in plasma NEFA, a change that may reflect lipolysis of increased TG-rich lipoproteins in circulation and fatty acid mobilization from adipose tissues due to increased adiposity and insulin resistance (12).

We found that olmesartan significantly decreased the high TGSR in ZF rats. Because this ARB remarkably ameliorates insulin resistance, it is reasonable to assume that the improvement of insulin sensitivity attenuates the overproduction of TG in these insulin-resistant animals. The determination of both insulin resistance (by FS-IVGTT) and TGSR (by the Triton method) can be difficult to perform in the same rats. To circumvent this obstacle, we roughly predicted the insulin resistance on the basis of the plasma insulin level. There was a high correlation between plasma insulin level and TGSR ($r = 0.927, P < 0.0001$) in ZF rats with and without olmesartan, suggesting that insulin resistance strongly regulates TG production. Despite the dramatic improvement of TG overproduction, we failed to find a significant reductive effect of ARB on the plasma TG. The decrease of TG production without any change in the plasma TG suggests a defect in TG catabolism. The marked reduction in blood pressure after olmesartan treatment might impair organic blood perfusion and consequently slow down the catabolic rate of TG in circulation. In a study by Mizuno et al. (18), 19 wk of olmesartan administration significantly decreased plasma TG in ZF rats. The 4-wk treatment of olmesartan in our present study might have been too short to elicit any observable hypotriglycerideremic action of ARBs. The olmesartan treatment decreased the NEFA level in ZF rats. We assume that the improved insulin resistance decreases the mobilization of fatty acids from the adipose tissues.

The liver TG content in the ZF rats was nearly 10 times higher than that in the control rats. Indeed, marked lipid droplets were seen in hepatocytes in these obese animals. Olmesartan treatment significantly halved the hepatic TG content and reduced lipid accumulation in the hepatocytes. To the best of our knowledge, this is the first study to demonstrate that ARB improves fatty liver. Insulin resistance and the compensatory hyperinsulinemia may be associated with the excessive TG accumulation in the liver (15). Enhancement in whole body insulin sensitivity decreased the availability of substrates for hepatic TG synthesis, such as glucose and free fatty acid, from peripheral tissues and the liver itself (5). In addition, blockade of the angiotensin II receptor may reverse the preferential shift from carbohydrate to β-oxidation of free fatty acids in an insulin-resistant state (7). ARBs have demonstrated multifunctional properties that go beyond their hemodynamic effects, especially as anti-inflammatory modulators and antioxidative stress agents (6). These effects may prevent the progression of hepatic steatosis (28). Toblli et al. (32) recently reported that enalapril, a kind of ACEI, exerted significant protective effect on hepatic steatosis in rats with nephrotic syndrome, suggesting that ARBs have a similarly favorable effect.

In summary, ARB decreased TG overproduction and liver TG accumulation while improving insulin sensitivity in insulin-resistant Zucker fatty rats. ARB might be favorable for dyslipidemia and fatty liver associated with insulin resistance.

**ACKNOWLEDGMENTS**

We are indebted to Sankyo Co. for providing olmesartan medoxomil.

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9. Kageyama A, Hirano T, Kageyama H, Oasaka T, Namba Y, Tsuji M, Kral JG, Lundholm K, Bjorntorp P, Sjostrom L, and Schersten T. Insulin resistance and the compensatory hyperinsulinemia may be associated with the excessive TG accumulation in the liver (15). Enhancement in whole body insulin sensitivity decreased the availability of substrates for hepatic TG synthesis, such as glucose and free fatty acid, from peripheral tissues and the liver itself (5). In addition, blockade of the angiotensin II receptor may reverse the preferential shift from carbohydrate to β-oxidation of free fatty acids in an insulin-resistant state (7). ARBs have demonstrated multifunctional properties that go beyond their hemodynamic effects, especially as anti-inflammatory modulators and antioxidative stress agents (6). These effects may prevent the progression of hepatic steatosis (28). Toblli et al. (32) recently reported that enalapril, a kind of ACEI, exerted significant protective effect on hepatic steatosis in rats with nephrotic syndrome, suggesting that ARBs have a similarly favorable effect.


