Effects of telmisartan and olmesartan on insulin sensitivity and renal function in spontaneously hypertensive rats fed a high fat diet

Hayato Yanagihara, Kentaro Ushijima, Yusuke Arakawa, Ken-ichi Aizawa, Akio Fujimura

Abstract
Although telmisartan, an angiotensin II receptor blocker (ARB), has an agonistic action for proliferator-activated receptor (PPAR)-γ in vitro, it remains to be determined whether telmisartan exerts such an action in vivo using a non-toxic dose (<5 mg/kg in rats). To address the issue, telmisartan (2 mg/kg) and olmesartan (2 mg/kg), another ARB without PPAR-γ agonistic action, were given to spontaneously hypertensive rats (SHR) fed a high fat diet (HFD). HFD decreased plasma adiponectin, and caused insulin resistance, hypertriglyceridemia and renal damage, which were improved by ARBs. Protective effects of telmisartan and olmesartan did not significantly differ. In addition, in vitro study showed that 1 μM of telmisartan did not elevate the mRNA expression of adipose protein 2, which is a PPAR-γ-stimulated adipogetic marker gene, in preadipocytes with 3% albumin. To obtain 1 μM of plasma concentration, oral dose of telmisartan was calculated to be 6 mg/kg, which indicates that PPAR-γ agonistic action is negligible with a non-toxic dose of telmisartan (<5 mg/kg) in rats. This study showed that 2 mg/kg of telmisartan and olmesartan ameliorated insulin resistance, hypertriglyceridemia and renal damage in SHR fed a HFD. As beneficial effects of telmisartan and olmesartan did not significantly differ, these were mediated through the PPAR-γ-independent actions.

1. Introduction
Hypertensive patients with insulin resistance and hyperlipidemia, which is known as the metabolic syndrome, have the increased risk of renal and cardiovascular diseases (1). Angiotensin II type 1 (AT1) receptor blocker (ARB) and angiotensin-converting enzyme inhibitor are reported to improve insulin resistance and delay the onset of diabetes in hypertensive patients (2).

Peroxisome proliferator-activated receptor (PPAR)-γ, a nuclear receptor mainly expressed in adipocytes, is involved in the regulation of glucose and lipid metabolism (3). Ligands of PPAR-γ improve the insulin sensitivity by a modulation of various gene expressions such as adiponectin (4,5). Telmisartan, an ARB, directly binds to the ligand-binding domain of PPAR-γ and can act as a PPAR-γ agonist (6). Therefore, it is anticipated that telmisartan provides a greater beneficial effect on glucose and lipid metabolism and consequently, a better protective effect against organ damages than other ARBs without PPAR-γ activating action do in hypertensive patients with the metabolic syndrome. Compatible with the idea, telmisartan (10 mg/kg) is reported to have a better renal protective effect than valsartan (10 mg/kg), another ARB, in a model of metabolic syndrome in rats (7). However, the maximal non-toxic dose of telmisartan is 5 mg/kg during a repeated dosing in rats (8), which indicates that 10 mg/kg of telmisartan had exerted a deleterious effect and modified pharmacologic profiles. Therefore, further studies using a non-toxic dose are needed to determine the protective effect of telmisartan against organ damages in rats with metabolic syndrome.

Olmesartan, an ARB, is widely used for the treatment of hypertension. Different from telmisartan, in vitro studies showed that olmesartan did not enhance PPAR-γ-mediated transcript activity (9), although the drug improved insulin sensitivity (10). In this study using a non-toxic dose, the protective effects of telmisartan against the insulin resistance, hypertriglyceridemia and renal damage were compared with those of olmesartan in spontaneously
hypertensive rats (SHR) fed a high fat diet, which is considered to be one of animal models of metabolic syndrome (7).

2. Materials and methods

1) In vivo study

2.1. Animals

Animal experiments were carried out in a humane manner after receiving approval from the Institutional Animal Experiment Committee of Jichi Medical University (13–216, 14–114, Tochigi, Japan), according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Animals were housed under a 12:12 h light/dark cycle at a room temperature of 24 ± 1 °C and humidity of 60 ± 10%. All animals were exposed to the light/dark cycle for at least 7 days before the experiment.

2.2. Study protocol

2.2.1. Experiment 1

Seven week-old male spontaneously hypertensive rats (SHR) were purchased from Japan SLC (Shizuoka, Japan). SHR were divided into five groups and fed a high fat diet (HFD) (D12492, 60% kcal as fat, Research Diets, New Brunswick, NJ) ad libitum. SHR were treated with vehicle (1% tragacanth gum solution), telmisartan (1 mg/kg), olmesartan (2 mg/kg) or olmesartan (2 mg/kg) once daily by gastric gavage. After more than 4 weeks-treatment, mean arterial blood pressure was measured for 24 h under a conscious state.

2.2.2. Experiment 2

Seven week-old male SHR were divided into four groups and fed either control fat diet (D12450J, 7% kcal as fat, Research Diets) or HFD. Groups 1 received control fat diet (control diet) and were treated with vehicle, 2 mg/kg telmisartan (HFD-Tel) or 2 mg/kg olmesartan (HFD-Olm) once daily for 11 weeks. Systolic blood pressure was also measured in all groups at every 5 weeks period. Insulin tolerance test was performed on week 9. Urine sample was collected for 24 h in a metabolic cage on week 11. At the end of the study, animals were anesthetized with pentobarbital sodium (50 mg/kg i.p.), and blood and kidney samples were graded from 0 to 4 according to the severity of the glomerular injury by one of authors (YA) blinded to the treatment protocol of the animals. At least 50 glomeruli in each sample were graded from 0 to 4 according to the severity of the glomerular sclerosis: 0 = normal, 1 = slight glomerular damage, the mesangial matrix and/or hyalinosis with focal adhesion, involving sclerosis of <25% of the glomerulus, 2 = sclerosis of 25–50%, 3 = sclerosis of 50–75%, and 4 = sclerosis of >75% of the glomerulus.

2.2.3. Experiment 3

Fifteen weeks-old male SHR were divided into five groups and fed either control fat diet or HFD. Groups 1 received control fat diet (control diet) and were treated with vehicle, 1 mg/kg telmisartan (HFD-Tel), 2 mg/kg telmisartan (HFD-Tel), olmesartan (2 mg/kg) or olmesartan (2 mg/kg) once daily by gastric gavage. After more than 4 weeks-treatment, mean arterial blood pressure was measured for 24 h under a conscious state.

2.3. Mean arterial blood pressure measurement

Rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.) and a polyethylene catheter (PE-50; Becton Dickinson, Franklin Lakes, NJ) filled with heparinized saline (10 U/ml, 0.05 ml/min) was inserted into the left common carotid artery and connected to a pressure transducer (P-3000S; Nidec Corp. Tokyo, Japan). Mean arterial blood pressure was recorded continuously in the animals under an alert and unrestrained condition, and with free access to food and water. Recording started more than 12 h after the surgery.

2.4. Insulin tolerance test

Rats were intraperitoneally injected with 0.75 U/kg of regular insulin without fasting. Blood was collected from the tail vein before and at 15, 30, 60 and 90 min after the injection.

2.5. Assays

Blood glucose concentration was measured using a Glutest Ace R (Sanwa Kagaku Kenkyusyo, Nagoya, Japan). Serum insulin and adiponectin concentrations were measured using commercialized enzyme-linked immunosorbent assay kits (Mercodia AB, Uppsala, Sweden; and R&D systems, Minneapolis, MN). The following formula was used to calculate the homeostasis model assessment for insulin resistance (HOMA-IR): [fasting insulin (µU/ml) × fasting glucose (mg/dl)]/405. Urinary albumin was measured by immunonephelometry. Urinary monocyte chemotactic protein-1 (MCP-1) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) concentrations were measured using enzyme immuno assay kits (IBL, Fujioka, Japan; and JalCA, Shizuoka, Japan). Triglycerid concentrations in serum were measured by triglyceride E-test kit based on glycerol-3-phosphate oxidase-DAOS method (Wako Pure Chemical Industries, Osaka, Japan).

2.6. Histopathological renal examination

Formalin fixed kidney sections were embedded in paraffin, cut into 5 μm thick sections and then stained with Periodic acid–Schiff staining for histopathological analysis. Samples were examined for glomerular injury by one of authors (YA) blinded to the treatment protocol of the animals. At least 50 glomeruli in each sample were graded from 0 to 4 according to the severity of the glomerular sclerosis: 0 = normal, 1 = slight glomerular damage, the mesangial matrix and/or hyalinosis with focal adhesion, involving sclerosis of <25% of the glomerulus, 2 = sclerosis of 25–50%, 3 = sclerosis of 50–75%, and 4 = sclerosis of >75% of the glomerulus.

2) In vitro study

2.7. Cells

3T3-L1 cells, the mouse preadipocyte, were maintained in DMEM supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin, Thermo Fisher Scientific) at 37 °C in a humidified 5% CO₂ atmosphere.

2.8. Adipocyte differentiation

For an induction of adipose differentiation, 3T3-L1 cells were seeded at 1.5 × 10^5 cells per well in 6-well culture plates and then were grown to confluence. Briefly, differentiation was induced by treating with 0.25 μM dexamethasone, 500 μM isobutyl methylxanthine and 1 μg/ml insulin in DMEM/FBS on day0. On day3, the medium was replaced with terminal differentiation medium (1 μg/ml insulin in DMEM/FBS). On day6, the medium was replaced with DMEM/FBS, and cells were fed for 2 days until assays (day 8).

i) Cells were exposed to 3.0% albumin and telmisartan (1, 10 or 100 μM).

ii) Cells were treated with telmisartan (10 μM) or olmesartan (10 μM).

Drugs were added continuously into culture media from the initiation of differentiation to the end of sample collection. In this study, PPAR-γ activity was evaluated by the mRNA expressions of adipose protein 2 (aP2) and adiponectine, adipogenic marker genes (11).
2.9. RNA extraction and real-time PCR

Total RNA from cells was extracted using the RNeasy Mini Kit (QIAGEN, Valencia, CA), following the manufacturer’s instructions, and was used for the preparation of cDNA using the PrimeScript RT reagent Kit (Takara Bio, Shiga, Japan). The quantification of gene expression level was performed by a real-time PCR using SYBR® Premix TaqII (Takara Bio). Specific primers for each gene were shown in Supplemental Table 1. Gene expression levels were normalized to Ribosomal protein S18 and were analyzed using the comparative threshold cycle method.

2.10. Calculation of a required dose of telmisartan

The present in vitro study with 3.0% albumin showed that PPAR-γ agonistic activity was not detected by 1 μM (515 ng/ml) of telmisartan, but by 10 μM (5150 ng/ml) of the drug. Assuming the pharmacokinetics of telmisartan can be described by the 1-compartment model, required dose (D) of telmisartan (mg/kg) to obtain 515 (or 5150) ng/ml of maximum concentration (Cmax) was calculated using follows equations (1) and (2).

\[
t_{max} = \frac{2.303}{ka - ke} \log \left( \frac{ka}{ke} \right)
\]

\[
C_{max} = \frac{F \times D}{Vd} \left( \frac{ka}{ke} \right)^{-\frac{Vd}{ka - ke}}
\]

ka: absorption constant, ke: elimination constant.

The following pharmacokinetic data about telmisartan are reported in rats: time to maximum concentration (tmax), 2 h; bioavailability (F), 66%; distribution of volume (Vd), 5.3 l/kg; and apparent clearance (Cl/f), 27 ml/min/kg (12).

2.11. Statistical analysis

Data are expressed as the mean ± SD. The repeated-measured ANOVA was used for the temporal data. Groups were compared by one-way ANOVA, and the difference between the two groups was analyzed by the Bonferroni–Dunn test. The p < 0.05 was considered to be significant.

3. Results

3.1. Effects of telmisartan and olmesartan on blood pressure

Repeated dosing of telmisartan (Fig. 1A) and olmesartan (Fig. 1B) dose-dependently decreased mean arterial blood pressure (MAP) in SHR. Differences in the area under the MAP-time course (AUC, mmHg hr) between the vehicle- and drug-groups were as follows; telmisartan 1 mg/kg: 708 ± 93, telmisartan 2 mg/kg: 1282 ± 175, olmesartan 1 mg/kg: 596 ± 62 and olmesartan 2 mg/kg: 1393 ± 230. As there were no significant differences in the AUC between telmisartan-1 mg/kg and olmesartan-1 mg/kg groups, or telmisartan-2 mg/kg and olmesartan-2 mg/kg groups, telmisartan 2 mg/kg and olmesartan 2 mg/kg were selected in the further experiments.

3.2. Effects of telmisartan and olmesartan on body weight, food intake and blood pressure

Compared to SHR with control diet, body weight was slightly, but not significantly increased in rats with a high fat diet alone (Fig. 2A). Telmisartan or olmesartan did not significantly influence body weight and food intake during 10 weeks (Fig. 2A,B). These drugs similarly decreased blood pressure in SHR (Fig. 2C).

3.3. Effects of telmisartan and olmesartan on glucose and lipid metabolism

After the injection of insulin, the decrease in blood glucose in rats with vehicle was significantly smaller than that in animals with control diet (Fig. 3A). However, there were no significant differences in the variable between the control and drug-treated groups.
Compared to the control diet group, the groups with a high fat diet had significantly lower plasma adiponectin concentration, which was similarly ameliorated by telmisartan and olmesartan (Fig. 3B).

At the end of the study, the values of HOMA-IR and plasma triglyceride concentration were significantly higher in the vehicle group, which were similarly corrected by telmisartan and olmesartan (Table 1).

3.4. Effects of telmisartan and olmesartan on urinary albumin and renal histopathology

Compared to the groups with control diet and vehicle alone, urinary albumin excretion significantly reduced in the groups with the drugs (Fig. 4A). High fat diet increased the urinary MCP-1 and 8-OHdG excretions (Fig. 4B,C), and caused glomerular injury, which were ameliorated by the drugs (Fig. 5, Table 2). Effects of telmisartan and olmesartan on these variables did not significantly differ.

3.5. Effect of telmisartan on mRNA expressions in 3T3-L1 adipocytes

Telmisartan concentration-dependently elevated the aP2 and adiponectin mRNA expressions in the adipocytes with 3.0% albumin (Fig. 6). Although 1 μM (515 ng/ml) of telmisartan at 3.0% albumin did not significantly elevated aP2 mRNA expression, the stimulating effect was detected at 10 μM (5150 ng/ml) of the drug. Under the condition with 3.0% albumin, only 100 μM of telmisartan significantly increased adiponectin mRNA expression. To obtain 515 and 5150 ng/ml of Cmax, oral doses of telmisartan were calculated to be 6 and 60 mg/kg, respectively.

Under the condition without additional albumin, 10 μM of telmisartan significantly elevated the aP2 and adiponectin mRNA expressions in the adipocytes, but olmesartan (10 μM) did not exert such stimulating effects (Fig. 7).

4. Discussion

To compare a blood pressure-unrelated effect of antihypertensive drugs, it is essential for selecting a dose of drug to obtain an identical blood pressure-lowering action. In this study, blood pressure-lowering effects of 2 mg/kg of telmisartan and 2 mg/kg of olmesartan did not significantly differ during the repeated dosing, and, therefore, 2 mg/kg/day of each drug was used in further studies.

As a maximal non-toxic dose of telmisartan is reported to be 5 mg/kg during a repeated dosing in rats [8], it is expected that...
Fig. 3. Effects of telmisartan (HFD-Tel) and olmesartan (HFD-Olm) for 9 weeks on insulin tolerance test (ITT) (A) and for 11 weeks on serum adiponectin concentration (B) in SHR fed a high fat diet (HFD). *p < 0.05, **p < 0.01 vs. control diet (CD), #p < 0.05 vs. HFD-vehicle, Mean ± SD, n = 6.

Table 1
Effects of telmisartan and olmesartan for 11 weeks on fasting blood glucose, insulin and triglyceride in SHR fed a high fat diet.

<table>
<thead>
<tr>
<th></th>
<th>Control diet</th>
<th>High fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Telmisartan</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>63.2 ± 5.4</td>
<td>77.6 ± 8.0</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>0.10 ± 0.01</td>
<td>0.32 ± 0.25</td>
</tr>
<tr>
<td>HOMA-IR 0.63 ± 0.21</td>
<td>35.9 ± 9.0</td>
<td>31.1 ± 7.1</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>21.1 ± 6.6</td>
<td>52.6 ± 30.7*</td>
</tr>
</tbody>
</table>

*p < 0.05 vs control diet, Mean ± SD, n = 6.

Fig. 4. Effects of telmisartan (HFD-Tel) and olmesartan (HFD-Olm) for 11 weeks on urinary excretions of albumin (A), MCP-1 (B) and 8-OHdG (C) in SHR fed a high fat diet (HFD). *p < 0.05, **p < 0.01 vs. control diet (CD), #p < 0.05, ##p < 0.01 vs. HFD-vehicle, Mean ± SD, n = 6.
2 mg/kg of telmisartan did not exert any deleterious effects. In this study with SHR fed a high fat diet, the increase of body weight and the amount of food intake did not significantly differ between vehicle group and 2 mg/kg of telmisartan group during the repeated dosing for 10 weeks under the present condition. On the other hand, 10 mg/kg of telmisartan was given to SHR fed a high fat diet for 10 weeks in the previous study (7). At the end of the experiment, body weight in the telmisartan-treated SHR was significantly lower than that of vehicle-treated rats, which suggests that 10 mg/kg of telmisartan affects the body weight gain in these animals.

In this study, telmisartan and olmesartan significantly improved the insulin resistance and hypertriglyceridemia, and ameliorated renal damage in SHR fed a high fat diet. In addition, such the effects of telmisartan did not significantly differ from those of olmesartan. Based on the data, it is speculated that the beneficial effects of telmisartan detected in this study were mediated through the PPAR-γ-independent actions under the present condition.

In this study with 3% albumin, 1 μM of telmisartan did not elevate the ap2 mRNA expression in 3T3-L1 adipocytes while 10 μM of the drug significantly elevated the variable. As PPAR-γ activity is reflected in the ap2 mRNA expression (11), these in vitro data suggest that 1 μM of telmisartan did not exert a PPAR-γ agonistic activity under the condition with 3% albumin. In general, a protein-unbound drug is involved in the expression of pharmacologic action. Our previous in vitro study showed that albumin concentration-dependently reduced the unbound fraction of telmisartan, and 16–26% of the drug was unbound at 3.0% albumin (submitted). Therefore, the unbound fractions of 1 and 10 μM of telmisartan at 3.0% albumin are calculated to be 0.16–0.26 μM and 1.6–2.6 μM, respectively. As the 50% effective concentration for PPAR-γ activation is reported to be 1–10 μM (9), 1 μM of telmisartan at 3.0% albumin might be too low to activate PPAR-γ. In addition, to obtain 1 μM (515 ng/ml) of plasma concentration (including unbound and bound fractions), oral dose of telmisartan was calculated to be 6 mg/kg, which is toxic dose (>5 mg/kg) in rats (8). Therefore, these data indicate that PPAR-γ agonistic action of telmisartan is negligible during a repeated dosing of a non-toxic dose of the drug (less than 5 mg/kg) in rats.

High fat diet augments oxidative stress in SHR (13), which, in turn, might reduce the secretion of adiponectin from adipocytes (14). These mechanisms can, at least in part, explain the decreased plasma adiponectin in SHR fed a high fat diet in this study. Adiponectin plays a major role in glucose and lipid metabolism, and has also anti-inflammatory effect (15). The decreased plasma adiponectin seems to be involved in the following findings in SHR fed a high fat diet in this study and other studies (16); 1) The reduced insulin sensitivity and increased plasma triglyceride, and 2) The enhanced renal inflammation reflected in the elevated urinary MIPC-1, and consequent renal damage.

Angiotensin II decreases plasma adiponectin concentration through the action on AT1 receptors (17), while ARB increases the variable (18). In addition, ARB diminishes the aldosterone-mediated suppression of adiponectin production by adipocytes.
In this study, although olmesartan lacks of PPAR-γ activating property, the drug elevated plasma adiponectin concentration in SHR fed a high fat diet, which is similar to telmisartan. Therefore, a blockade of AT1 receptor would be involved in the PPAR-γ-independent pathways for the elevating plasma adiponectin concentration by telmisartan and olmesartan in this study.

In this study, telmisartan and olmesartan similarly elevated plasma adiponectin concentration in SHR fed a high fat diet, and the high fat diet-induced changes were improved as follows:

1) Correction of insulin resistance:

Adiponectin activates AMP-activated protein kinase, which inhibits hepatic gluconeogenesis and increases muscle glucose transport (15), and consequently, improves insulin resistance.

2) Decrease in plasma triglyceride concentration:

CD-36 (fatty acid transporter), acyl-coenzyme A and uncoupling protein-2 are involved in fatty acid transport and oxidation (15). Adiponectin can stimulate these proteins in muscle and increase fat combustion and energy waste (15, 20), and consequently, decreases plasma triglyceride.

3) Amelioration of renal damage:

MCP-1 is involved in the recruitment of monocytes and regulation of migration and infiltration of monocytes/macrophages, and it causes inflammation and tissue damage (21). Adiponectin decreases MCP-1 expression in tissues (22), and consequently, ameliorates renal damage. Blood pressure-lowering action of ARBs also contributed to the renal protective effect of these drugs. Angiotensin II up-regulates NADPH oxidase which produces reactive oxygen species (ROS) (23). In addition, it is suggested that an increased production of ROS contributes to diabetic complications (24). Previous study showed that ARBs might suppress NADPH oxidase-mediated ROS production via AT1 receptor (25), which might be a potential pathway involved in PPAR-γ-independent pathways. In this study, elevated urinary excretion of 8-OHdG by a high fat diet, a marker of oxidative DNA damage, was ameliorated by telmisartan and olmesartan. Based on these findings, AT1 receptor blockade-mediated reduction in ROS production would be involved in the PPAR-γ-independent pathways linking to the beneficial effects of these drugs.

In summary, this study showed that telmisartan and olmesartan improved insulin resistance, corrected hypertriglyceridemia and
ameliorated renal damage in SHR fed a high fat diet after a repeated dosing of under the maximal non-toxic dose (<5 mg/kg). As the beneficial effects of telmisartan did not significantly differ from those of olmesartan, these were mediated through the PPAR-γ-independent actions involving a blockade of AT1 receptor, under the present condition.

Conflict of interest

The authors declared no conflict of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jphs.2016.06.003.

References