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Full paper

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

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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 adipogenic 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 type1 (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

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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), telmisartan (2 mg/kg), olmesartan (1 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. Groups 2–4 received HFD and was orally treated with vehicle (HFD-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 using a standard tail-cuff sphygmomanometer (KN-201, Natsume, Tokyo, Japan) in an awake state throughout the experimental 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 collected after fasting for 12 h.

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 Copal Electronics Co., 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 chemoattractant 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). Triglyceride 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% CO_2 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).