Original Article

Telmisartan but Not Candesartan Affects Adiponectin Expression *In Vivo* and *In Vitro*

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To examine the effects of telmisartan on peroxisome proliferator–activated receptor γ activation, we compared the effects of telmisartan with those of candesartan on adipocytokines and glucose and lipid metabolism *in vivo* and *in vitro*. *In vivo*, 56 patients with both type 2 diabetes and hypertension were enrolled and randomized to receive either telmisartan (40 mg) or candesartan (8 mg) for 3 months. Serum adiponectin, HbA1c levels, lipid profiles and blood pressure were recorded at the beginning and 3 months later. *In vitro*, differentiated 3T3-L1 adipocytes were treated with telmisartan, candesartan, pioglitazone or vehicle for 24 h, and then adiponectin mRNA and protein levels were measured. The results showed that most of the metabolic parameters, including the lipid profiles, did not change significantly during the study in either group. However, the changes in serum adiponectin and plasma glucose over 3 months were significantly greater in the telmisartan group than in the candesartan group. *In vitro*, although the protein level of adiponectin was not significantly elevated, the mRNA expression of adiponectin was elevated 1.5-fold by telmisartan in 3T3-L1 adipocytes. Our findings suggest that telmisartan may have beneficial effects in type 2 diabetes beyond its antihypertensive effect. (*Hypertens Res* 2008; 31: 601–606)

***Key Words*: telmisartan, candesartan, adiponectin, peroxisome proliferator–activated receptor γ**

Introduction

Angiotensin type 1 receptor (AT1R) blockers (ARBs) prevent the binding of angiotensin II to the AT1R and are widely used for the treatment of hypertension. Recently, several clinical trials have revealed a reduced risk for the development of type 2 diabetes in patients treated with ARBs and angiotensin-converting enzyme inhibitors (ACEIs) (*1–4*). Since ramipril conferred only marginal protection against the development of type 2 diabetes, whereas rosiglitazone showed significant effects in the same study (*5*, *6*), peroxisome proliferator–activated receptor (PPAR) γ activation has been proposed to exert important roles in the prevention of type 2 diabetes; although the precise mechanisms remain unclear. Two ARBs, telmisartan and irbesartan, appear to have PPAR γ activating activities (*7*, *8*). Therefore, these two agents may have additional favorable metabolic effects beyond renin-aldosterone–system blockade.

Adiponectin is a hormone secreted exclusively by adipocytes, and its production is regulated by PPAR γ activation. Adiponectin is widely recognized as a key protein regulating insulin resistance and the metabolic syndrome (*9*, *10*). Within a clinical setting, it is important to determine whether ARBs increase adiponectin by acting as partial PPAR γ agonists. It has been reported that telmisartan has the strongest PPAR γ affinity, whereas candesartan has the lowest affinity (*11*). Therefore, in this study, we investigated the effects of two ARBs, telmisartan and candesartan, on adiponectin, glucose metabolism and lipid profiles *in vivo* in Japanese patients with

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both type 2 diabetes and hypertension, and the effect *in vitro* on adiponectin expression.

Methods

In Vivo Experiment

The patients with type 2 diabetes and essential hypertension were enrolled at Kitasato Institute Hospital between August 2003 and September 2005. Exclusion criteria included: 1) age older than 75 years; 2) hemoglobin A1c (HbA1c) >8.0%; 3) previous use of ARBs or ACEIs; 4) serum creatinine >1.5 mg/dL or aspartate aminotransferase (AST)/alanine aminotransferase (ALT) elevation (3 times upper limit of normal); and 5) change in agents taken for diabetes, hypertension, or hyperlipidemia within the previous 3 months. Fifty-six patients (45 men and 11 women; mean age, 59.4±11.7 years) were enrolled and randomly assigned to two groups: 1) a group receiving telmisartan 40 mg per day, or 2) a group receiving candesartan 8 mg per day. The patients were instructed to adhere to the standard diet and regular daily exercise throughout the study. We did not change agents for diabetes, hypertension, or hyperlipidemia during the study period.

Body mass index (BMI) and blood pressure were recorded, and serum levels of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG), glucose, IRI, and adiponectin were measured at the start of the study and 3 months later. Serum concentrations of total adiponectin were measured by sandwich ELISA (Otsuka Pharmaceutical, Tokyo, Japan; Fujirebio, Tokyo, Japan) (*12*). As 12 patients used insulin injection and insulin is known to affect adiponectin expression and secretion (*13*, *14*), we measured the serum adiponectin and insulin levels in the 44 patients who did not inject insulin.

All patients received an explanation of the procedures and possible disadvantages of the study and provided their written informed consent before participating. This study was performed in conformity with the Declaration of Helsinki and was approved by our institutional review board.

Table 1. Baseline Characteristics and Metabolic Variables of Study Subjects

Variables	Reagent	
	Telmisartan (n=28)	Candesartan (n=28)
Age (year)	57.9±11.5	60.8±12.0
Men/women	23/5	22/6
SBP (mmHg)	150.5±16.4	156.3±13.4
DBP (mmHg)	88.3±16.0	88.9±11.6
FPG (mg/dL)	141±27	121±18
IRI (μU/mL)*	19.1±12.3	11.0±5.6
HOMA-R*	7.2±5.0	3.3±1.6
HbA1c (%)	6.6±1.0	6.9±1.1
BMI (kg/m^2)	25.0±3.2	25.0±4.5
TG (mg/dL)	178.0±114.2	189.2±132.6
HDL-C (mg/dL)	56.1±12.7	55.7±11.3
LDL-C (mg/dL)	143.5±34.8	134.5±40.6
Adiponectin (μg/mL)*	6.3±2.7	7.7±3.7
Number of anti-diabetic agents users		
Insulin	6	6
Sulfonylurea	11	12
Biguanides	2	2
Thiazolidines	0	0
α-GI	2	1
Glinides	1	1
Number of antihypertensive agents users		
CCB	7	6
α-Blockers	0	1
β-Blockers	0	0
Diuretics	0	0
ACEIs	0	0
Number of anti-dyslipidemic agents users		
Statins	10	11
Fibrates	3	2

Values are expressed as number (n) or mean±SD. *n=22 in both group. SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; IRI, immuno-reacitve insulin; HOMA-R, homeostasis model assessment for insulin resistance; BMI, body mass index; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; α-GI, α-glucosidase inhibitor; CCB, calcium channel blocker; ACEIs, angiotensin converting enzyme inhibitor.

In Vitro Experiment

The 3T3-L1 preadipocytes were cultured and differentiated with a standard differentiation mixture (dexamethasone, 3-isobutyl-1-methylxanthine [IBMX], insulin, and 10% FBS) as previously described (*15*). The differentiated adipocytes were stained by oil-red–staining to confirm fat accumulation after culture for 9 d. Telmisartan was provided by Nippon Boehringer Ingelheim (Tokyo, Japan), and candesartan and pioglitazone were provided by Takeda Pharmaceutical (Osaka, Japan).

The differentiated adipocytes were washed, incubated for 16 h without serum, and cultured with vehicle or 10 μmol/L of telmisartan, candesartan, or pioglitazone for 24 h. As for the dosage of agents, we followed previous studies (*7*, *8*, *16*). After 24-h culture, the supernatants were collected and cultured cells were homogenized to obtain RNA.

The concentration of adiponectin in supernatants was measured using a mouse/rat adiponectin ELISA kit (Otsuka Pharmaceutical). Adiponectin mRNA expression in 3T3-L1 cells was analyzed by real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR) using a Bio-Rad iCycler system (Bio-Rad, Hercules, USA), as previously

Table 2. Clinical and Laboratory Values before and after Treatment for 3 Months

Variables	Telmisartan			Candesartan			*p* value**
	Before	After	*p* value*	Before	After	*p* value*	
SBP (mmHg)	150.5±16.4	136.9±14.9	<0.05	156.3±13.4	139.8±16.6*	<0.05	n.s.
DBP (mmHg)	88.3±16.0	80.6±10.1	<0.05	88.9±11.6	80.6±9.9*	<0.05	n.s.
HbA1c (%)	6.6±1.0	6.6±0.9	n.s.	6.9±1.1	6.8±1.1	n.s.	n.s.
IRI (μU/mL)	19.1±12.3	13.9±7.1	n.s.	11.0±5.6	13.4±11.5	n.s.	n.s.
BMI (kg/m^2)	25.0±3.2	25.0±3.4	n.s.	25.0±4.5	24.5±4.4	n.s.	n.s.
TG (mg/dL)	169.9±107.4	199.4±169.0	n.s.	210.7±149.6	189.3±117.1	n.s.	n.s.
HDL-C (mg/dL)	56.1±12.7	55.3±13.9	n.s.	55.7±11.3	56.1±11.6	n.s.	n.s.
LDL-C (mg/dL)	143.5±34.8	135.2±27.3	n.s.	134.5±40.6	143.2±40.5	n.s.	n.s.

Values are expressed as mean±SD. *Student's *t*-test used for within-group changes. **Unpaired *t*-test to compare between group differences (telmisartan *vs* candesartan). SBP, systolic blood pressure; DBP, diastolic blood pressure; IRI, immuno-reacitve insulin; BMI, body mass index; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

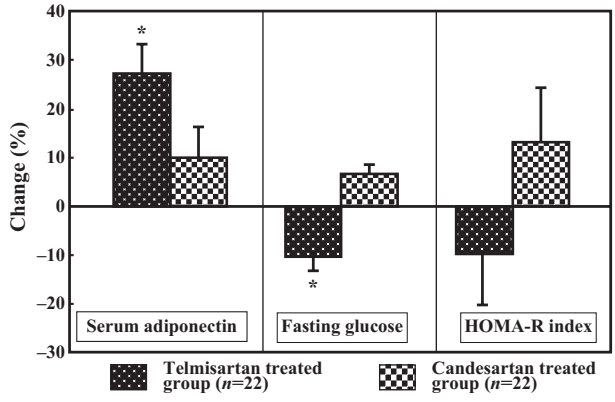

Fig. 1. *Changes in serum adiponectin levels, fasting glucose and HOMA-R during 3 months of ARB administration. The changes in the telmisartan group (adiponectin: from 6.3±2.7 to 7.9±3.1 μg/mL; glucose: from 141±27 to 118±8 mg/dL; HOMA-R: from 7.2±5.0 to 4.1±2.2) were greater than those in the candesartan group (adiponectin: from 7.7±3.7 to 8.7±5.3 μg/mL; glucose: from 121±18 to 129±18 mg/dL; HOMA-R: from 3.3±1.6 to 4.4±3.6). *p<0.05 comparing with candesartan group. Values are expressed as the means±SD.*

described (*17*). The probe and primer sets were as follows: probe, 5′-FAM-cataagcggcttctccaggctctcct-TAMRA-3′; forward primer, 5′-tgttggaatgacaggagctgaa-3′; and reverse primer, 5′-cacactgaagcctgagcgatac-3′.

Statistical Analysis

Data are presented as the means±SD for the *in vivo* study and means±SEM for the *in vitro* study. The levels of adiponectin protein and mRNA expression were compared by one-way analysis of variance, with Scheffe's post hoc test. Student's paired *t*-test was used for comparisons of variables between before and after medications. Unpaired *t*-tests were performed for comparisons of variables between the two groups. Values of $p<0.05$ were recognized as statistically significant. All analyses were conducted using StatView software (Version 5.01; SAS Institute, Cary, USA).

Results

In Vivo Experiments

The baseline characteristics of the patients are shown in Table 1. No significant differences were noted between the two groups. As previously reported, the serum adiponectin level was negatively correlated with homeostasis model assessment of insulin resistance (HOMA-R) ($r=-0.52$, $p<0.05$) (*18*).

At 3 months, both telmisartan and candesartan lowered systolic blood pressure (SBP) and diastolic blood pressure (DBP), and the changes in blood pressure were not different between the two groups. Most of the metabolic parameters did not change significantly during the study period (Table 2). However, the changes in serum adiponectin and plasma glucose levels after 3 months were significantly greater in the telmisartan group than in the candesartan group (Fig.1, $p<0.05$).

In Vitro Experiments

Telmisartan Induced mRNA Expression of Adiponectin in 3T3-L1 Cells

The culture experiments with 3T3-L1 adipocytes showed that the culture with telmisartan significantly increased mRNA expression of adiponectin ($p<0.05$), whereas that with candesartan did not. However, the PPAR γ ligand, pioglitazone, stimulated adiponectin expression to a significantly greater extent than did negative control ($p<0.01$) (Fig. 2).

However, the concentration of adiponectin in the supernatant was unchanged with telmisartan and with candesartan, but was significantly increased with pioglitazone ($p<0.005$) (Fig. 3).

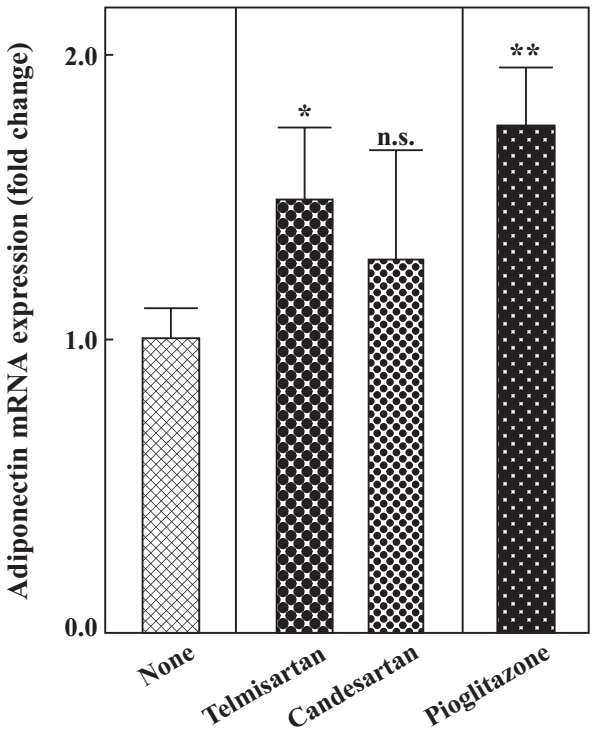

Fig. 2. *Adiponectin mRNA expression. Telmisartan (10 μmol/L) and pioglitazone (10 μmol/L) up-regulated adiponectin gene expression in differentiated 3T3-L1 adipocytes (day 9). The relative mRNA expressions are shown. Data are expressed as the means±SEM from quadruplicate experiments. *$p < 0.05$, **$p < 0.01$ comparing with negative control (ANOVA with Scheffe's post hoc test).*

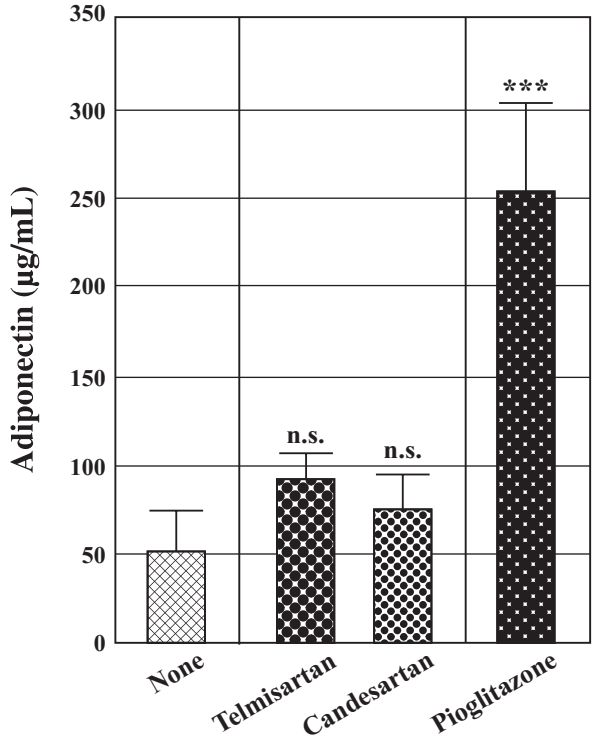

Fig. 3. *Adiponectin secretion in the supernatant of 3T3-L1 adipocytes after incubation with telmisartan (10 μmol/L) or pioglitazone (10 μmol/L). Telmisartan (10 μmol/L) did not increase the adiponectin protein level in the 3T3-L1 supernatant. Data are expressed as the means±SEM from quadruplicate experiments. ***$p < 0.005$ comparing with negative control (ANOVA with Scheffe's post hoc test).*

Discussion

This study revealed that telmisartan more effectively elevated serum adiponectin than candesartan. Furthermore, we have also demonstrated that adiponectin was increased at least in part by the direct action of telmisartan on adipocytes.

Previously, several ARBs were reported to increase adiponectin in clinical studies (*19*, *20*). It was shown valsartan elevated adiponectin in patients with hypertension and diabetes but not in patients with hypertension only (*19*). On the other hand, candesartan was reported to increase adiponectin in patients with hypertension (*20*). Therefore, ARBs may have adiponectin-elevating activity as a class effect. However, this study showed that telmisartan and candesartan were similar in their hypotensive action but not in their adiponectin elevation in patients with diabetes and hypertension. Therefore, telmisartan should have certain effects beyond the renin-angiotensin–system blockade. Since telmisartan was reported to have the strongest PPAR γ affinity among ARBs and candesartan was reported to show the lowest affinity (*11*), and Clasen *et al*. reported that PPAR γ–activating ARBs induced adiponectin expression (*21*), we believe that the difference between the effects of telmisartan and candesartan on adiponectin expression observed in this study was induced by PPAR γ activation.

Our results are supported by three non-randomized studies (*22–24*) and are concordant with five randomized studies (*25–29*). Miura *et al*. reported an elevation of adiponectin in 18 subjects upon changing their treatment from candesartan or valsartan to telmisartan (*22*). Pershadsingh and Kurtz (*23*) presented a case report in which insulin sensitivity was altered by a change of ARB from valsartan to telmisartan. Recently, Mori *et al*. (*24*) reported that telmisartan improves lipid metabolism and adiponectin production in Japanese hypertensive patients with type 2 diabetes. However, these were not randomized prospective studies. Previously, three randomized studies showed an improvement of insulin sensitivity by telmisartan (*25–27*). Although these studies did not show a significant elevation of adiponectin, the authors agreed that telmisartan activated PPAR γ. The differences in adiponectin elevation between these studies and ours might

be explained by the number of study subjects. Benndorf *et al*. (*25*) enrolled 37 hypertensive patients randomized to three groups of 12 patients, while Nagel *et al*. (*26*) enrolled 20 obese subjects and used a cross-over study design. In the latter study, the adiponectin elevation was not statistically significant, but the *p* value was 0.09. This trend in elevation might be meaningful. Although Vitale *et al*. (*27*) showed that telmisartan improved the glucose tolerance in patients with metabolic syndrome, whereas losartan did not, they did not report the adiponectin concentration. Although Negro *et al*. (*28*) and Derosa *et al*. (*29*) reported that telmisartan elevated adiponectin, irbesartan, which was used as a control agent, also increased adiponectin. Because irbesartan is also known as a PPAR γ activating ARB (*8*), they could not definitively determine whether the adiponectin elevation was an ARB class effect or a specific effect of irbesartan as a PPAR γ activating ARB. Therefore, to the best of our knowledge, our study is the first randomized clinical report to show that telmisartan increased adiponectin levels more than other ARBs in Japanese patients.

In vitro, we showed that telmisartan upregulated the mRNA expression of adiponectin. However, adiponectin production in the supernatant was not increased by telmisartan. This discrepancy between the mRNA and protein might suggest that the stimulation of adiponectin expression by telmisartan acts mainly on a transcriptional rather than a secretory level. Fujimoto *et al*. (*30*) reported that telmisartan elevated the mRNA level of adiponectin in 3T3-L1 adipocytes. However, in their study, the protein level of adiponectin was not evaluated. In adipocytes, insulin modulates protein processing and secretion independent of transcription (*13*). As with insulin, telmisartan and PPAR γ might modulate adipocyte responses through differentiation, transcription, protein processing, and secretion independently. Identification of co-activators and co-repressors of PPAR γ would clarify the role of telmisartan as a metabolic modifier.

In conclusion, our findings suggest that telmisartan may have beneficial effects in type 2 diabetes and the metabolic syndrome beyond its antihypertensive effects.

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