Original Article

Inhibition of Vascular Angiotensin-Converting Enzyme by Telmisartan *via* the Peroxisome Proliferator–Activated Receptor γ Agonistic Property in Rats

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The angiotensin receptor blocker (ARB) telmisartan is a partial agonist of peroxisome proliferator–activated receptor γ (PPARγ). Typical PPARγ agonists suppress the gene expression of angiotensin-converting enzyme (ACE) in vascular tissues. However, it remains unclear whether or not PPARγ activation by telmisartan can inhibit vascular ACE activity. We compared the effects of PPARγ agonistic telmisartan and non-agonistic valsartan on ACE, vascular function and oxidative stress in stroke-prone spontaneously hypertensive rats (SHR-SP) and in sodium (1% NaCl)-loaded SHR-SP. SHR-SP and sodium-loaded SHR-SP received placebo, 1 mg/kg telmisartan, or 10 mg/kg valsartan for 2 weeks. Systolic blood pressure (SBP) was equally reduced in SHR-SP given either telmisartan or valsartan compared with SHR-SP given placebo. However, neither telmisartan nor valsartan suppressed SBP in sodium-loaded SHR-SP. Acetylcholine induced significantly less vasorelaxation in SHR-SP than in Wistar-Kyoto rats, but telmisartan and valsartan each significantly prevented such vasorelaxation. However, telmisartan significantly attenuated acetylcholine-induced vasorelaxation in sodium-loaded SHR-SP, whereas valsartan did not. Telmisartan significantly attenuated NADPH oxidase subunit $p22^{phox}$ gene expression in both SHR-SP and sodium-loaded SHR-SP, whereas valsartan did not. Likewise, telmisartan also significantly attenuated the significantly increased vascular ACE activity in sodium-loaded SHR-SP, whereas valsartan did not. In conclusion, the partial PPARγ agonist telmisartan might inhibit vascular ACE activity, and result in the prevention of oxidative stress and endothelial dysfunction more effectively than non-agonistic valsartan. (*Hypertens Res* 2007; 30: 1231–1237)

***Key Words*: angiotensin-converting enzyme, angiotensin receptor blocker, oxidative stress, peroxisome proliferator–activated receptor γ, vasorelaxation**

Introduction

Angiotensin II plays an important role in increasing blood pressure by stimulating angiotensin II type 1 (AT_1) receptors. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are widely applied in the clinical setting: the former inhibits angiotensin II formation, whereas the latter blocks angiotensin II binding to AT_1 receptors. Angiotensin II also induces growth factors and cytokines as well as other factors that play important roles in cardiovascular diseases (*1*). Therefore, the inhibition of angiotensin II function by ACE inhibitors or ARBs has been useful for lowering blood pressure and preventing cardiovascular diseases.

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Table 1. Effects of Telmisartan and Valsartan on Body Weight, Heart Weight and Heart Weight to Body Weight

Parameters	WKY rats (n=6)	SHR-SP			1% salt-diet SHR-SP		
		Placebo (n=6)	Telmisartan (n=6)	Valsartan (n=6)	Placebo (n=9)	Telmisartan (n=8)	Valsartan (n=8)
Body weight (g)	373±5**,##	289±10	304±3	301±5	222±17**	299±4##	294±5##
Heart weight (mg)	97±2**	119±3	110±1	120±4	105±6*	115±2	119±2#
Heart weight/body weight (mg/g)	2.60±0.03*	4.35±0.18	3.63±0.05**	3.98±0.09**	4.80±0.14**	3.84±0.06##	4.06±0.08##

*p<0.05 and **p<0.01 *vs*. placebo (SHR-SP). #p<0.05 and ##p<0.01 *vs*. placebo (1% salt-diet SHR-SP). SHR-SP, stroke-prone spontaneously hypertensive rats; WKY, Wistar-Kyoto.

Various ARBs have been widely applied to treat clinical hypertension. Although ARBs block AT_1 receptors, some ARBs such as telmisartan and irbesartan are also partial agonists of peroxisome proliferator–activated receptor-γ (PPARγ) (*1*, *2*). Among the ARBs, telmisartan is the most potent PPARγ agonist (*1*, *2*). In contrast, ARBs such as valsartan have no PPARγ agonistic effect (*1*). In general, PPARγ plays a crucial role in regulating gene transcription in insulin-sensitive tissues, thereby enhancing insulin sensitivity (*3*). Toba *et al*. (*4*) reported that PPARγ ligands, pioglitazone and bezafibrate, prevent the streptozotocin-induced increase in vascular ACE gene expression and protein content in rats. However, it remains unclear whether or not telmisartan has more effective vascular ACE activity than other ARBs that have no PPARγ agonistic effect.

We compared the effects of telmisartan (PPAR-γ partial agonist) with valsartan (no PPARγ agonist effect) on vascular remodeling in stroke-prone spontaneously hypertensive rats (SHR-SP). Our preliminary study showed that telmisartan (1 mg/kg per day) and valsartan (10 mg/kg per day) exerted equally hypotensive effects in SHR-SP for 2 weeks. Here, we evaluated whether or not PPARγ activation by telmisartan attenuates vascular ACE activity.

Methods

Animals

Twelve-week-old male Wistar-Kyoto (WKY) rats and SHR-SP were obtained from Japan SLC Inc. (Shizuoka, Japan). The experiments proceeded in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College).

SHR-SP received an oral placebo, 1 mg/kg telmisartan or 10 mg/kg valsartan daily for 2 weeks. Sodium-loaded SHR-SP received an oral placebo, 1 mg/kg telmisartan or 10 mg/kg valsartan daily for 2 weeks. All rats including control WKY rats had free access to water containing 1% NaCl. Systolic blood pressure (SBP) was monitored by tail-cuff plethysmography (BP-98, Softron Co., Tokyo, Japan). After placebo or ARBs were administered for 2 weeks, the rats were weighed and then anesthetized with 35 mg/kg of sodium pentobarbital i.p. to obtain blood and tissues.

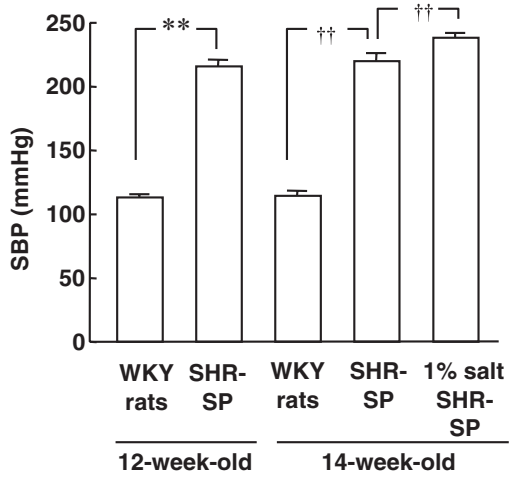

Fig. 1. *SBP in 12-week-old WKY rats and SHR-SP and in 14-week-old WKY rats, SHR-SP and SHR-SP loaded with 1% salt. **p<0.01 vs. 12-week-old SHR-SP. ††p<0.01 vs. 14-week-old SHR-SP.*

Plasma ACE Activity, Plasma Renin Activity and Angiotensin II Concentration

Plasma was separated from the blood samples by centrifugation at 3,000 rpm for 15 min at 4°C. Plasma renin activity (PRA) was determined using an SRL renin kit (TFB Co., Tokyo, Japan). Plasma ACE activity was measured using a synthetic substrate, hippril-His-Leu, specifically designed to detect ACE (Peptide Institute, Inc., Osaka, Japan) (*5*). Angiotensin II concentrations were measured using an enzyme immunoassay kit (Peninsula Laboratories Inc., Belmont, USA).

Acetylcholine-Induced Vasorelaxation in Isolated Rat Artery

Isolated rat carotid arteries were cut into 10 × 1.0 mm helical strips and placed on a myograph under a resting tension of 1.0 g (*6*) in Tyrode's solution (137 mmol/L NaCl, 2.7 mmol/L KCl, 1.8 mmol/L $CaCl_2$, 1.1 mmol/L $MgCl_2$, 0.42 mmol/L NaH_2PO_4, 12 mmol/L $NaHCO_3$ and 5.7 mmol/L glucose, pH 7.4; bathing medium) at 37°C and continuously bubbled with 5% CO_2 in O_2.

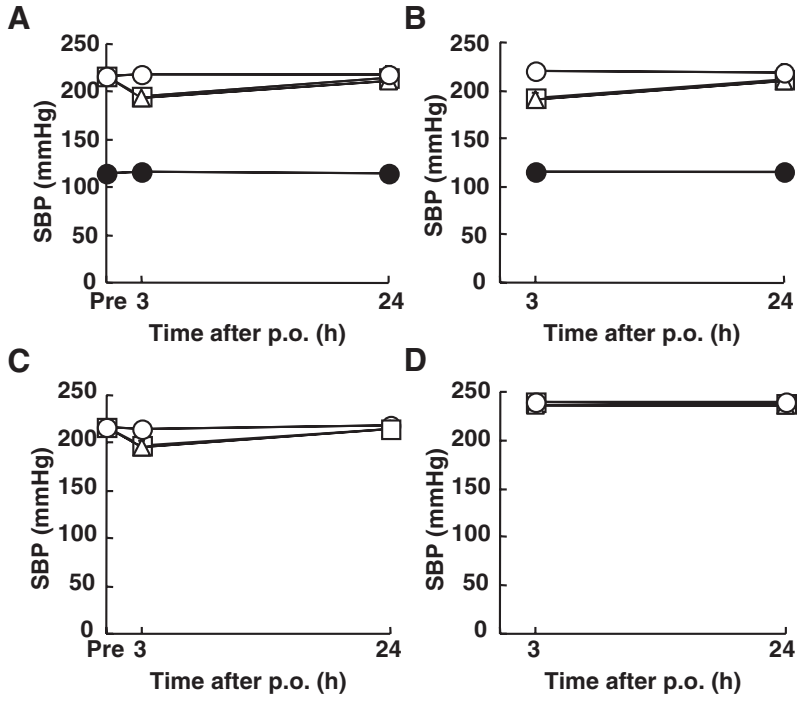

Fig. 2. *SBP before (Pre) and 3 and 24 h after first (A) and last (B) administrations of placebo (open circles), telmisartan (open triangles), and valsartan (open squares) in SHR-SP and in WKY rats (closed circles). SBP before (Pre) and 3 and 24 h after first (C) and last (D) administrations of placebo (open circles), telmisartan (open triangles), and valsartan (open squares) in sodium-loaded SHR-SP.*

The strips were initially vasoconstricted to 50 mmol/L KCl, and then the bathing medium was washed out. Relaxation induced by acetylcholine was assessed after contraction to a steady-state tension using 1 μmol/L norepinephrine.

Reverse Transcription–Polymerase Chain Reaction

Total RNA (5 μg) was extracted from the aortae using Trizol (Life Technologies, Rockville, USA) in water treated with 0.1% diethyl pyrocarbonate and reverse-transcribed using SuperScript II reverse transcriptase and oligo$(dT)_{12–18}$ primer (Invitrogen, Carlsbad, USA). The reaction proceeded in first-strand buffer, 1 mmol/L dNTPs and 20 mol/L dithiothreitol at 42°C for 50 min. The polymerase chain reaction (PCR) mixture contained 1 μL of the cDNA reaction, 20 pmol/L primers, PCR buffer, 0.4 mmol/L dNTPs, and 2.5 U *Taq* polymerase. The reaction proceeded in a RoboCycler (Stratagene, La Jolla, USA). The sequences of the PCR primers for $p22^{phox}$ amplification were: sense, 5′-GCTCATCTGTCT GCTGGAGTA-3′ and antisense, 5′-ACGACCTCATCTGTC ACTGGA-3′; AT_1 receptor: sense, 5′-GCACACTGGCAA TGTAATGC-3′ and antisense, 5′-GTTGAACAGAAC AAGTGACC-3′; and for β-actin: sense, 5′-CCAAGGCCA ACCGCGAGAAGATGAC-3′ and antisense, 5′-AGGGTA CATGGTGGTGCCGCCAGAC-3′. We used β-actin to calibrate sample loading. The PCR products were separated by electrophoresis on 2% agarose gels stained with ethidium bromide and visualized using ultraviolet transillumination (*7*).

Vascular ACE Activity

Vascular tissues were minced and homogenized in 5 vol (w/v) of 20 mmol/L Tris-HCl buffer, pH 8.3, containing 5 mmol/L $Mg(CH_3COO)_2$, 30 mmol/L KCl, 250 mmol/L sucrose, and 0.5% Nonidet P-40 (*8*). The ACE activity in the supernatant was measured by incubation with hippuryl-His-Leu.

Statistical Methods

Data are expressed as means±SEM. Statistical analyses were performed using a parametric test with Fisher's protected least significant difference. Values of $p<0.05$ were considered to indicate statistical significance.

Results

Body and Heart Weight

Table 1 shows the effects of telmisartan and valsartan on the weight of the body, brain, heart and kidneys. The placebo-treated SHR-SP groups weighed significantly less than WKY

Table 2. Effects of Telmisartan and Valsartan on Plasma Renin Activity (PRA), Plasma ACE Activity and Serum Angiotensin II Concentration (Serum AII Conc.)

Parameters	WKY rats (n=6)	SHR-SP			1% salt-diet SHR-SP		
		Placebo (n=6)	Telmisartan (n=6)	Valsartan (n=6)	Placebo (n=9)	Telmisartan (n=8)	Valsartan (n=8)
PRA (ng AI/mL per h)	7.2±0.7	8.4±0.7	22.3±3.4**	14.9±1.3*	11.1±0.9	18.0±2.0##	16.3±1.7#
Plasma ACE activity (mU/mL)	103±2.9**	44.3±1.5	46.0±1.2	46.9±1.0	46.0±1.0	47.2±0.8	45.9±1.3
Serum AII conc. (pg/mL)	18.0±0.9	15.7±0.7	36.7±3.2**	25.3±1.9*	18.3±1.6	31.1±4.0##	27.9±1.5#

AI and AII, angiotensin I and angiotensin II; ACE, angiotensin-converting enzyme; SHR-SP, stroke-prone spontaneously hypertensive rats; WKY, Wistar-Kyoto. *p<0.05 and **p<0.01 *vs*. placebo (SHR-SP). ##p<0.01 and ##p<0.01 *vs*. placebo (1% salt-diet SHR-SP).

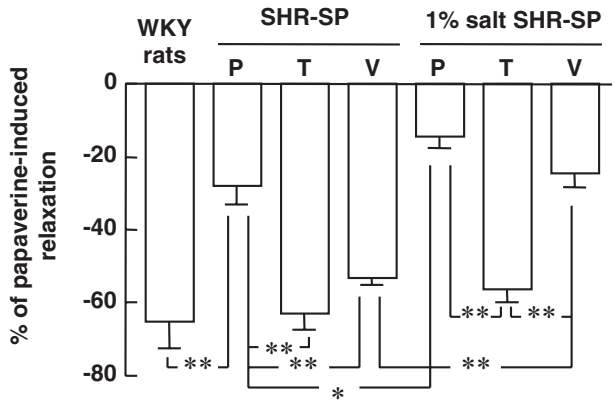

Fig. 3. *Acetylcholine-induced vasorelaxation of carotid arteries contracted with noradrenaline in WKY rats, placebo (P)-, telmisartan (T)-, or valsartan (V)-treated SHR-SP and placebo (P)-, telmisartan (T)-, or valsartan (V)-treated sodium-loaded SHR-SP. Results are shown as ratios (%) of maximal relaxation induced by papaverine. *p<0.05 and **p<0.01.*

rats, and salt-loaded SHR-SP weighed significantly less than SHR-SP. Body weight in each of the three SHR-SP groups did not significantly differ, whereas salt-loaded SHR-SP administered with telmisartan or valsartan weighed significantly more than the groups given placebo. On the other hand, the ratio of heart weight to body weight in placebo-treated SHR-SP was significantly greater than in WKY rats and significantly lower in both telmisartan- and valsartan-treated SHR-SP than in the placebo-treated SHR-SP. Furthermore, the ratio in placebo-treated, sodium-loaded SHR-SP was higher than in placebo-treated SHR-SP, but that in sodium-loaded SHR-SP groups administered with telmisartan or valsartan was also significantly lower than in the placebo group.

Blood Pressure

SBP was significantly higher in SHR-SP than in WKY rats at 12 weeks of age (Fig. 1). The SBP in SHR-SP was slightly higher at 14 than at 12 weeks of age, but the difference was not significant. On the other hand, SBP in SHR-SP that were sodium-loaded for 2 weeks was significantly higher than that in the age-matched SHR-SP.

Figure 2A and B show the effects of telmisartan and valsartan on SBP in SHR-SP. SBP 3 h after the first doses of telmisartan and valsartan were significantly lower than the control value, but gradually recovered within 24 h. After the last dose, both ARBs exerted the same level of hypotensive effects at 3 h, but not at 24 h. Overall, telmisartan and valsartan had similar hypotensive effects. Figure 2C and D show the effects of telmisartan and valsartan on SBP in sodium-loaded SHR-SP. The SBP 3 h after the first doses of telmisartan and valsartan were significantly lower than the control value, but the SBP gradually recovered within 24 h. However, even at 3 h after the last administration, neither of the ARBs exerted any hypotensive effects.

Plasma ACE Activity, PRA, and Angiotensin II Concentration

Table 2 shows the PRA, plasma ACE activities, and angiotensin II concentrations. The PRA and angiotensin II concentrations were significantly higher in the telmisartan- and valsartan-treated groups than in placebo-treated, sodium-loaded SHR-SP. On the other hand, plasma ACE activity did not differ among any of the SHR-SP or sodium-loaded SHR-SP groups.

Vascular Responses

Acetylcholine induced vasorelaxation in all rats (Fig. 3), but to a lesser extent in the placebo-treated SHR-SP than in the WKY rats. Vasorelaxation was significantly greater in the telmisartan- and valsartan-treated groups than in the placebo-treated group. The amount of vasorelaxation did not significantly differ between the telmisartan- and valsartan-treated groups. Vasorelaxation was significantly lower in the placebo-treated, sodium-loaded group than in the placebo-treated group. Vasorelaxation in telmisartan-treated sodium-loaded SHR-SP was significantly more obvious than in the groups given either placebo or valsartan, and that in the latter two groups did not significantly differ.

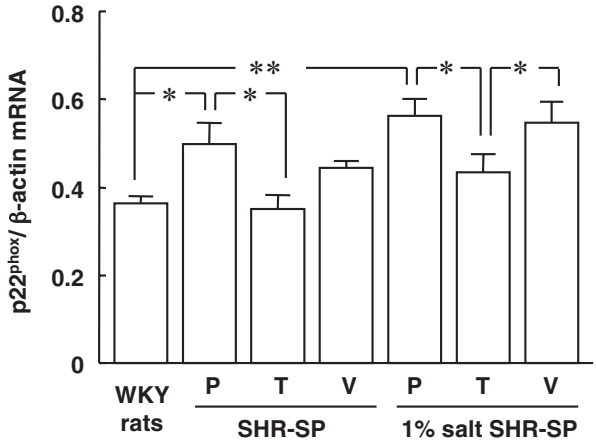

Fig. 4. *Ratios of p22^{phox} expression to β-actin in aortae obtained from WKY rats, placebo (P)-, telmisartan (T)-, or valsartan (V)-treated SHR-SP and placebo (P)-, telmisartan (T)-, valsartan (V)-treated sodium-loaded SHR-SP. *p < 0.05 and **p < 0.01.*

Vascular p22^{phox} Expression

Figure 4 shows p22^{phox} expression in the vascular tissues. Placebo-treated SHR-SP expressed significantly more p22^{phox} than WKY rats. However, the level of p22^{phox} expression in SHR-SP given telmisartan was significantly lower than in those given either placebo or valsartan. Placebo-treated, sodium-loaded SHR-SP expressed significantly more p22^{phox} than placebo-treated SHR-SP. Telmisartan-treated, sodium-loaded SHR-SP expressed significantly less p22^{phox} than placebo- and valsartan-treated SHR-SP, and the levels did not significantly differ between the latter two groups.

Vascular ACE Activity and AT_1 Receptor Expression

Figure 5A shows levels of vascular ACE activity levels. The level was significantly higher in placebo-treated SHR-SP than in WKY rats, and in placebo-treated, sodium-loaded SHR-SP than in placebo-treated SHR-SP. The level tended to be lower in the telmisartan-treated group than in placebo, but not in the valsartan-treated group. On the other hand, ACE activity in the telmisartan-treated, sodium-loaded SHR-SP was significantly lower than that in the groups given either placebo or valsartan, and the latter two groups did not significantly differ from each other.

Figure 5B shows the levels of vascular AT_1 receptor expression. These levels did not differ significantly among WKY rats, placebo-treated SHR-SP, and placebo-treated, sodium-loaded SHR-SP. However, levels in SHR-SP given either telmisartan or valsartan were significantly lower than in those given placebo. On the other hand, levels among placebo-, telmisartan-, and valsartan-treated sodium-loaded SHR-SP did not significantly differ from each other.

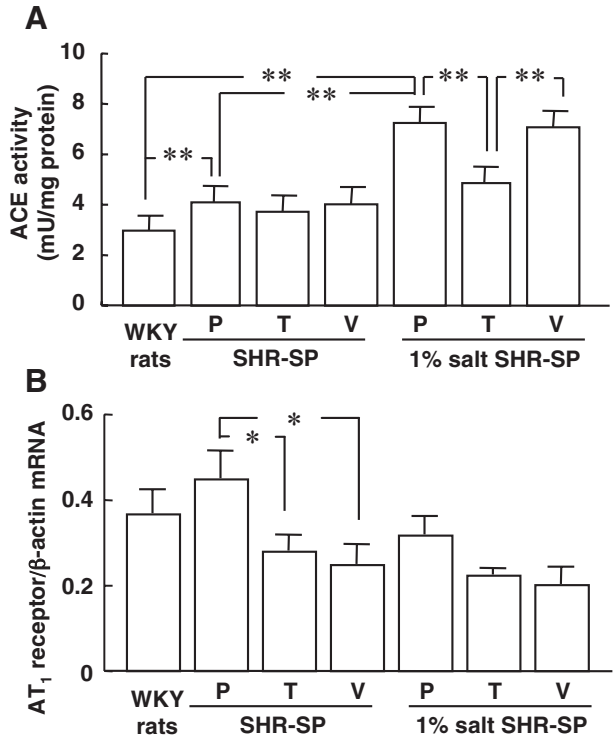

Fig. 5. *ACE activities in WKY rats, placebo (P)-, telmisartan (T)-, or valsartan (V)-treated SHR-SP, and placebo (P)-, telmisartan (T)-, or valsartan (V)-treated sodium-loaded SHR-SP (A). Ratios of AT_1 receptor expression to β-actin in aortae obtained from WKY rats, placebo (P)-, telmisartan (T)-, or valsartan (V)-treated SHR-SP and placebo (P)-, telmisartan (T)-, or valsartan (V)-treated sodium-loaded SHR-SP (B). *p < 0.05 and **p < 0.01.*

Discussion

The present study compared the effects of telmisartan and valsartan on vascular RAS. Telmisartan is the most potent PPARγ agonist among ARBs whereas valsartan has no PPARγ agonistic effect. As our preliminary study found, telmisartan (1 mg/kg per day) and valsartan (10 mg/kg per day) exerted equal hypotensive effects for 2 weeks in SHR-SP. However, in sodium-loaded SHR-SP, telmisartan and valsartan exerted similar hypotensive effects at 3 h after the first administration but not at 3 h after the last administration. Therefore, 1 mg/kg of telmisartan and 10 mg/kg of valsartan exerted similar hypotensive effects in SHR-SP but not in sodium-loaded SHR-SP. Overall, these doses of ARBs similarly affected blood pressure. Neither ARB attenuated plasma ACE activity in SHR-SP or sodium-loaded SHR-SP. On the other hand, PRA and angiotensin II concentrations in both ARB-treated groups were significantly higher than in placebo-treated SHR-SP or placebo-treated, sodium-loaded

SHR-SP. However, vascular ACE activity was significantly lower in sodium-loaded SHR-SP administered telmisartan than in that administered valsartan. These findings suggest that the partial PPARγ agonist telmisartan might inhibit vascular ACE activity.

Not only adipocytes and monocyte/macrophages, but also vascular smooth muscle and endothelial cells express PPARγ (*9*). We found significantly more vascular ACE activity in SHR-SP than in WKY rats, and in sodium-loaded SHR-SP than in SHR-SP. On the other hand, plasma ACE activity did not significantly differ among WKY rats, SHR-SP, and sodium-loaded SHR-SP. These results support previous findings (*10*, *11*). Therefore, blood pressure might have more influence in vascular than in plasma ACE-dependent angiotensin II formation (*11*). Toba *et al*. (*4*) recently demonstrated that PPARγ ligands, pioglitazone and bezafibrate prevent streptozotocin-induced increases in vascular ACE gene expression and protein content in rats. We found here that the partial PPARγ agonist telmisartan significantly reduced ACE activity in sodium-loaded SHR-SP, whereas the non-PPARγ agonist valsartan did not. Other reports have shown that PPARγ ligands significantly reduce blood pressure in hypertensive rats without diabetes or obesity (*12–14*). These findings suggest that telmisartan exerts its hypotensive effect through vascular ACE inhibition *via* the PPARγ agonistic effect.

PPARγ can not only improve insulin sensitivity but it also prevents endothelial dysfunction in humans (*15*). We showed here that both telmisartan and valsartan significantly augmented acetylcholine-induced vascular relaxation in SHR-SP, whereas only telmisartan exerted this effect in sodium-loaded SHR-SP. Both ARBs equally reduced SBP in SHR-SP, but not sodium-loaded SHR-SP. Therefore, the mechanism through which telmisartan prevents endothelial dysfunction in sodium-loaded SHR-SP is apparently unrelated to its effect on blood pressure. Oxidative stress has been thought to play an important role in endothelial dysfunction, and expression of the NAD(P)H oxidase subunit $p22^{phox}$ is regarded as a typical marker of such stress (*16–18*). One study has shown that a PPARγ ligand inhibits expression of the NADPH oxidase subunits $p22^{phox}$ and $p47^{phox}$ *in vitro* (*19*). Another study has shown that PPARγ ligands inhibit NADPH oxidase expression in the streptozotocin-induced diabetic rat aorta *in vivo* (*4*). Here, $p22^{phox}$ expression was significantly reduced in the aortae of telmisartan-treated SHR-SP and sodium-loaded SHR-SP compared with placebo-treated rats, whereas valsartan did not attenuate $p22^{phox}$ expression. Therefore, endothelial dysfunction might be prevented through the reduction of $p22^{phox}$ expression *via* the PPARγ agonistic effect.

On the other hand, an increase in $p22^{phox}$ expression reflects an increase in NAD(P)H oxidase activity, which is closely related to oxidative stress caused by angiotensin II in vascular tissues (*20*). We showed here that not only ACE activity but also $p22^{phox}$ expression was significantly higher in the aortae of SHR-SP and sodium-loaded SHR-SP than in those of WKY rats. We previously reported that ACE activity is significantly higher in the aortae of SHR-SP than WKY rats, and even more so in those of sodium-loaded SHR-SP than in SHR-SP (*21*). In genetically hypertensive models, such as SHR and SHR-SP, ACE expression in the vasculature is upregulated and angiotensin II action against blood vessels is strengthened (*10*, *22*). The present study found that telmisartan, but not valsartan, reduced ACE activity *via* its PPARγ agonistic effect, and this might result in a decrease of angiotensin II action including NAD(P)H oxidase in the vascular tissues.

Cardiac hypertrophy was evident in all SHR-SP and sodium-loaded SHR-SP. However, both telmisartan- and valsartan-treated SHR-SP were equally and significantly less hypertrophic than either the placebo-treated SHR-SP or the sodium-treated SHR-SP. Left ventricular weight significantly and positively correlates with the tissue angiotensin II concentration in SHR (*23*). Diep *et al*. (*24*) reported that long-term administration of a PPARγ ligand in SHR-SP significantly reduces inflammatory markers such as nuclear factor-κB and tumor necrosis factor-α, but not cardiac hypertrophy. The present study found that telmisartan does not prevent cardiac hypertrophy any more effectively than valsartan. The doses of telmisartan and valsartan used herein might exert similar levels of AT_1 receptor antagonism in cardiac tissues, and telmisartan's PPARγ agonistic effect might contribute to vascular remodeling rather than to cardiac hypertrophy.

In the present study, we did not obtain direct data on telmisartan-induced PPARγ agonistic effects, such as reduction of adipocyte size. Recently, Sugimoto *et al*. (*25*) reported that telmisartan (5 mg/kg per day) reduced the accumulation of visceral fat and decreased adipocyte size to a much greater extent than did valsartan (5 mg/kg per day) in rats fed a high fat, high-carbohydrate diet. More recently, Mori *et al*. (*26*) demonstrated that in Otsuka Long Evans Tokushima Fatty rats, telmisartan administration (5 mg/kg per day) resulted in significantly lower adipocyte size when compared with valsartan (10 mg/kg per day), which was given at the same dose as in the present study. The telmisartan dose of 1 mg/kg per day in the present study may have weakly stimulated PPARγ. A typical PPARγ ligand, pyoglitazone, significantly reduced vascular ACE activity in a N^{ω}-nitro-L-arginine methyl ester–induced hypertensive model (*27*), and the dose of telmisartan also reduced vascular ACE activity in the present study. On the other hand, Imayama *et al*. (*28*) demonstrated that pyoglitazone and telmisartan, a partial PPARγ agonist, significantly reduced AT_1 receptor expression in cultured rat vascular smooth muscle cells, but that the non-PPARγ agonists olmesartan and candesartan did not. Although this finding was confirmed in the *in vitro* study, we observed that both telmisartan and valsartan reduced vascular AT_1 receptor expression in SHR-SP, but that there were no significant differences in reducing AT_1 receptor expression between telmisartan and valsartan. The telmisartan dose used in the present study may have been insufficient to exert the full PPARγ agonistic effects. Further studies using higher doses of telmisartan are thus needed.

In conclusion, telmisartan, which has PPARγ agonistic properties, inhibited vascular ACE activity in rats more effectively than valsartan, which does not have PPARγ agonistic properties. Therefore, telmisartan might prevent vascular remodeling more effectively than other ARBs.

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