Telmisartan Reduces Mortality and Left Ventricular Hypertrophy With Sympathoinhibition in Rats with Hypertension and Heart Failure

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BACKGROUND
Angiotensin II type 1 receptor (AT₁R) blockers have various benefits on hypertension and/or heart failure. We demonstrated that telmisartan (TLM), an AT₁R blocker, causes sympathoinhibition by reduction of reactive oxygen species (ROS) in the rostral ventrolateral medulla (RVLM) of stroke-prone spontaneously hypertensive rats (SHRSPs). The aim of this study was to determine whether TLM improves survival in rats with hypertension and heart failure.

METHODS
Angiotensin II–infused and salt-loaded SHRSPs were divided into TLM-treated, candesartan cilexetil (CAN)–treated, and control groups. We determined the dose of TLM or CAN with similar depressor effects. We examined survival, urinary norepinephrine excretion (uNE) as a parameter of sympathoexcitation, ROS in the RVLM, and left ventricular (LV) end-diastolic pressure (LVEDP). LV hypertrophy (LVH) was assessed by echocardiography and heart/body weight.

RESULTS
Compared with the control group, TLM improved survival to a greater extent than CAN. At 4 weeks after treatment, ROS in the RVLM and uNE were significantly lower in the TLM-treated group than in the CAN-treated group, despite the similar depressor effects. At 8 weeks after the treatments, LVH and LVEDP were attenuated in the TLM-treated group compared with the CAN-treated group.

CONCLUSIONS
Our results suggest that TLM has the potential to reduce mortality, LVH, and LVEDP and that enhanced sympathoinhibition by reduction of ROS in the RVLM might be one of the mechanisms contributing to the beneficial actions of TLM in a model of rats with severe hypertension and heart failure.

Keywords: ARB; blood pressure; brain; heart failure; hypertension; sympathetic nerve activity.

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In hypertension and hypertension-induced cardiovascular diseases, lowering blood pressure reduces cardiovascular events. Cardiac hypertrophy associated with hypertension is not only a physiologically adaptive state before heart failure but also an independent risk factor of major cardiac events. Although there are many factors that can induce cardiac hypertrophy, pressure overload state is a major hypertrophy-inducing factor. Angiotensin II and angiotensin II type 1 receptor (AT₁R) blockers (ARBs) are clinically available as a highly effective and well-tolerated class of agents in the treatment for hypertension. Among the depressor agents, AT₁R blockers (ARBs) are clinically available as a highly effective and well-tolerated class of agents in the treatment for hypertension. In addition, it has been suggested that ARBs effectively prevent cardiac hypertrophy and improve the cardiovascular outcomes in patients with hypertension, left ventricular (LV) dysfunction, acute myocardial infarction, and/or heart failure. Although antihypertrophic effects were not significantly different among ARBs, the degrees of regression of cardiac hypertrophy were different between ARBs in basic research under a similarly hypertensive stimulation. Several clinical trials have already demonstrated the benefits of ARBs in heart failure with systolic dysfunction. However, the CHARM-Preserved and I-PRESERVE study, which was conducted in patients with preserved LV ejection fraction, showed that cardiovascular event did not differ between groups. It has not been fully determined whether the effects of ARBs on heart failure with LV hypertrophy (LVH) were class effects of ARBs or not.

Abnormal sympathoexcitation is involved in the pathogenesis of heart failure, and sympathoinhibition has been considered to have prominent benefits in the treatment for heart failure and/or LVH. Previous studies have determined that brain AT₁R-induced reactive oxygen species (ROS) causes sympathoexcitation in hypertensive rats and animal models with heart failure and that systemic
administered ARBs could act on brain AT_1R directly, thereby causing sympathoinhibition.\textsuperscript{23,24} We also have demonstrated that oral administration for 28 days of telmisartan (TLM), one of the ARBs, could cause sympathoinhibition by reduction of ROS in the rostral ventrolateral medulla (RVLM), known as a vasomotor center, of stroke-prone spontaneously hypertensive rats (SHRSPs) and that the sympathoinhibition was significantly greater in TLM-treated than in candesartan cilexetil (CAN)–treated SHRSPs.\textsuperscript{25} These results are supported by a previous report, which indicated that blockade of brain AT_1R is different between different orally administered ARBs.\textsuperscript{26} However, it has not been determined whether the beneficial effects on heart failure and/or LVH are related to the blockade of brain AT_1R by orally administered ARBs.

Considering these backgrounds, the aim of this study was to determine whether TLM could improve survival in a rat model of severe hypertension and heart failure. For this purpose, we used angiotensin II–infused and salt-loaded SHRSPs as a new severe hypertension and heart failure model, dividing them into a TLM-treated group, a CAN–treated group, and a vehicle (VEH)–treated group.

**METHODS**

**Animals**

This study was reviewed and approved by the committee on ethics of Animal Experiments, Kyushu University Graduate School of Medical Sciences, and conducted according to the Guidelines for Animal Experiments of Kyushu University. SHRSPs were fed a 0.3% sodium chloride (NaCl) diet until 8 weeks of age, and then they were switched to an 8% NaCl diet. Eleven-week-old SHRSPs, fed a high-salt diet and subcutaneously infused with angiotensin II (200 ng/kg per minute) by osmotic minipump (Alzet, Cupertino, CA) to the end of the experiments, were divided into 3 groups, given either VEH (0.5% methylcellulose), TLM (2 mg/kg/day dissolved in 0.5% methylcellulose; Sigma Aldrich, St. Louis, MO), or CAN (2 mg/kg/day dissolved in 0.5% methylcellulose; Sigma Aldrich) by oral gavage once daily, and evaluated for survival. The doses of TLM and CAN were selected based on our earlier report so that they would exhibit comparable antihypertensive effects.\textsuperscript{25} Moreover, LV pressure, ROS in the RVLM, and heart/body weight were evaluated in other TLM–, CAN–, or VEH-treated rats for 4, 6, and 8 weeks.

**Measurement of blood pressure, heart rate and urinary norepinephrine excretion**

Systolic blood pressure and heart rate were measured daily by tail-cuff method (BP-90A; Softron, Tokyo, Japan). We calculated urinary norepinephrine excretion for 24 hours as a parameter of sympathoexcitation, as described in our previous studies.\textsuperscript{18,19,22,25}

**Evaluation of LV diameter and LVH**

To evaluate LV diameter and LVH, LV systolic diameter, LV diastolic diameter, interventricular septum thickness, and LV posterior wall thickness were measured by echocardiography at 4, 6, and 8 weeks after the treatments under light sodium pentobarbital anesthesia with spontaneous respiration. An echocardiography system (SSD5000; Aloka, Tokyo, Japan) with a dynamically focused 7.5 MHz linear array transducer was used, and M-mode tracings from the short-axis view at the level of the papillary muscle were recorded. Moreover, heart/body weight were measured as the parameter of LVH.

**Measurement of LV end-diastolic pressure**

To measure LV end-diastolic pressure (LVEDP), a catheter was inserted into the left ventricle through the right common carotid artery in rats in a pentobarbital-anesthetized condition at 4, 6, and 8 weeks after the treatments.

**Measurement of thiobarbituric acid–reactive substances in the RVLM**

Thiobarbituric acid–reactive substance levels in the RVLM were measured as a parameter of ROS at 4, 6, and 8 weeks after treatment, as described in our previous studies.\textsuperscript{18,19}

**Statistics**

All values are expressed as mean ± SEM. Survival analysis was performed by the Kaplan–Meier method, and between-group difference in survival was tested by the log-rank test. Comparisons between any 2 mean values were performed using Bonferroni’s correction for multiple comparisons. Analysis of variance was used to compare all of the parameters in TLM–, CAN–, and VEH-treated SHRSPs. Differences were considered to be statistically significant at \( P < 0.05 \).

**RESULTS**

**Survival**

Figure 1 shows survival in all groups. We have checked for a visible brain bleeding in each rat that died, and rats with visible brain bleeding were excluded. The number of rats that had visible brain bleeding in the TLM-treated group and the CAN-treated group was the same (\( n = 6 \) for each). We considered the rats with massive pleural effusion and ascites as death from heart failure, and 20 rats that survived in each group were finally examined. Compared with VEH, TLM improved survival to a greater extent than CAN (Figure 1). At 11 weeks after the treatments, only TLM-treated SHRSPs survived.

**Blood pressure, heart rate, and urinary norepinephrine excretion**

Baseline systolic blood pressure and heart rate were similar in TLM–, CAN–, and VEH-treated SHRSPs (Table 1). Systolic blood pressure was similar in TLM– and CAN-treated SHRSPs throughout the experiments and was significantly lower in TLM– and CAN-treated SHRSPs than in VEH-treated SHRSPs at 6 weeks after treatment (Table 1). Heart
rate was not different in CAN- and VEH-treated SHRSPs at 6 weeks after treatment and was significantly lower in TLM-treated SHRSPs than in CAN-treated SHRSPs at 6 and 8 weeks after treatment (Table 1), similar to the results of each treatment at 4 weeks, as shown in our previous study.25

Urinary norepinephrine excretion was similar in CAN- and VEH-treated SHRSPs at 4 and 6 weeks after the treatments (Figure 2a,b). However, urinary norepinephrine excretion was significantly lower in TLM-treated SHRSPs than in those of VEH-treated SHRSPs at 4 and 6 weeks after the treatments (Figure 2a,b). Throughout the experiments, urinary norepinephrine excretion was significantly lower in TLM-treated SHRSPs than in CAN-treated SHRSPs (Figure 2a–c).

ROS in the RVLM

Thiobarbituric acid–reactive substance levels in the RVLM were similar in CAN- and VEH-treated SHRSPs at 4 and 6 weeks after the treatments (Figure 3a,b) and were significantly lower in TLM-treated SHRSPs than in CAN-treated SHRSPs during the experiments (Figure 3a–c). However, thiobarbituric acid–reactive substance levels in the RVLM were similar in CAN- and VEH-treated SHRSPs at 4 and 6 weeks after the treatments (Figure 3a,b).

LV diameter and LVH

Table 2 shows the results of echocardiography and heart/body weight. LV systolic diameter was significantly smaller both in TLM- and CAN-treated SHRSPs than in VEH-treated SHRSPs at 6 weeks after treatment. However, LV systolic diameter was significantly smaller in TLM-treated SHRSPs than in CAN-treated SHRSPs at 8 weeks after treatment. LV diastolic diameter was similar in TLM- and CAN-treated SHRSPs throughout the experiments.

Interventricular septum thickness plus LV posterior wall thickness and heart/body weight were significantly smaller both in TLM- and CAN-treated SHRSPs than in VEH-treated SHRSPs at 6 weeks after treatment. However, interventricular septum thickness plus LV posterior wall thickness and heart/body weight were significantly smaller in TLM-treated SHRSPs than in CAN-treated SHRSPs at 8 weeks after treatment.

LV end-diastolic pressure

LVEDP was significantly lower both in TLM- and CAN-treated SHRSPs than in VEH-treated SHRSPs at 4 and 6 weeks after treatment (Figure 4a,b). However, LVEDP was significantly lower in TLM-treated SHRSPs than in CAN-treated SHRSPs at 6 and 8 weeks after treatment (Figure 4b,c).

DISCUSSION

Major novel findings in this study are as follows. First, TLM improved survival in angiotensin II–infused and
Benefits of Telmisartan on Survival and LVH

First, TLM exerted a greater depressor effect on the blood pressure of salt-loaded SHRSPs to a greater extent than CAN, despite the similar depressor effects. Second, TLM reduced ROS in the RVLM, urinary norepinephrine excretion, LVEDP, and LVH in angiotensin II–infused and salt-loaded SHRSPs to a greater extent than CAN, despite the similar depressor effects. Third, TLM-induced sympathoinhibition occurred before the reduction of LVH and LVEDP in angiotensin II–infused and salt-loaded SHRSPs. From these results, we
Kishi et al. concluded that TLM has the potential to reduce mortality, LVH, and LVEDP and that enhanced sympathoinhibition by reduction of ROS in the RVLM might be one of the mechanisms contributing to the beneficial actions of TLM in an animal model with severe hypertension and heart failure. Moreover, the beneficial effects of TLM might not be class effects of ARBs.

The most novel finding of this study is that ARBs could improve the survival of rats with hypertension and heart failure and that the beneficial effects were different between TLM and CAN. In clinical studies, the benefits of ARBs on cardiovascular events in heart failure have already been established. However, it has not been clarified whether these benefits were class effects of ARBs and independent of their depressor effects. We demonstrated that both TLM and CAN significantly improved survival, with strong and similar depressor effects in rats with hypertension and heart failure. Furthermore, the improvement of survival was prominent in the TLM-treated group to a greater extent than in the CAN-treated group in spite of the similar depressor effects. Interestingly, reduction of LVH was also determined in the TLM-treated group to a greater extent than in the CAN-treated group despite the similar depressor effects. LVH is known as a predictive factor for the mortality of heart failure associated with hypertension, and we consider that the improvement in survival would be associated with the reduction of LVH. These results could suggest that ARBs have significant benefits on survival in hypertension and heart failure and that the benefits might be obtained independent of the depressor effects.

We must focus on the mechanisms, probably associated with the reduction of LVH, by which TLM could obtain the better results in survival than CAN. Although our experimental data showed a less significant regression of

### Table 2. Echocardiographic data and heart/body weight

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weeks</th>
<th>No.</th>
<th>LVDD, mm</th>
<th>LVDs, mm</th>
<th>IVS+PW, mm</th>
<th>HW/BW, mg/g</th>
</tr>
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<tbody>
<tr>
<td>VEH</td>
<td>6</td>
<td>8</td>
<td>6.8±0.5</td>
<td>4.0±0.4</td>
<td>4.8±0.5</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>CAN</td>
<td>6</td>
<td>20</td>
<td>6.2±0.4*</td>
<td>3.6±0.5*</td>
<td>4.3±0.4*</td>
<td>3.1±0.4*</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>10</td>
<td>7.0±0.6</td>
<td>4.1±0.4</td>
<td>4.6±0.5</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>TLM</td>
<td>6</td>
<td>20</td>
<td>6.1±0.6*</td>
<td>3.6±0.5*</td>
<td>4.2±0.5*</td>
<td>3.0±0.3*</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>17</td>
<td>6.8±0.5</td>
<td>3.8±0.3**</td>
<td>4.3±0.4**</td>
<td>3.2±0.2**</td>
</tr>
</tbody>
</table>

Abbreviations: CAN, candesartan cilexetil; HW/BW, heart weight/body weight; IVS, interventricular septum thickness; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; PW, posterior wall thickness; TLM, telmisartan.

*P < 0.05 vs. VEH at 6 weeks; **P < 0.05 vs. CAN at 8 weeks.

Figure 4. Left ventricular end-diastolic pressure in vehicle (VEH)-treated, candesartan cilexetil (CAN)-treated, and telmisartan (TLM)-treated groups of angiotensin II-infused and salt-loaded stroke-prone spontaneously hypertensive rats at (a) 4 weeks (n = 5 for each group), (b) 6 weeks (n = 5 for each group), and (c) 8 weeks (n = 5 for CAN and TLM) after treatment. *P < 0.05 vs. VEH, **P < 0.05 vs. CAN in TLM.
pressure overload–induced cardiac hypertrophy by CAN than by TLM, clinical trials revealed that ARBs can markedly decrease the cardiovascular death or hospital admission of patients with chronic heart failure.7–11,13 In this study, TLM, not CAN, caused sympathoinhibition in angiotensin II–infused and salt-loaded SHRSPs. Furthermore, at 6 and 8 weeks after treatment, the changes in heart rate showed opposite direction in the TLM-treated group and the CAN-treated group, probably because of the sympathoinhibition by TLM. These results are quite similar to those of our previous studies.23,25 Sympathetic nerve activity is mainly determined by the neural activity of the RVLM in hypertension.27 It has been demonstrated that brain oxidative stress causes hypertension through sympathoexcitation.18,19,23,25,28 We have demonstrated that AT1R-induced ROS in the RVLM causes sympathoexcitation in hypertensive rats.19,23,25 Direct blockade of AT1R in the RVLM causes sympathoinhibition in hypertensive rats.19 Peripherally administered ARBs also inhibit the central actions of angiotensin II in the brain.23–26 In our previous studies, TLM could blockade brain AT1R, whereas CAN could not.25 After peripheral administration, TLM penetrates the blood–brain barrier in a dose- and time-dependent manner to inhibit centrally mediated effects of angiotensin II; this is because of the high lipophilic property of TLM.26 In a previous study with salt-loaded SHRSPs, angiotensin II strikingly increased brain oxidative stress, and CAN attenuated the increase in brain oxidative stress and brain damage.29 Although we used only 1 dose and did not examine the dose dependency in TLM or CAN, we consider that orally administered TLM (2 mg/kg/day) can penetrate the blood–brain barrier and reach the RVLM of SHRSPs, whereas CAN (2 mg/kg/day) cannot sufficiently do this. We consider that the beneficial effect of ARBs on survival of hypertension and heart failure rats might be due to sympathoinhibition by reduction of ROS in the RVLM. To strengthen our consideration, it should be clarified whether interventions modulating ROS in the RVLM could have the similar benefits in rats with hypertension and heart failure.

We should also discuss the effects of ARBs on cardiomyocyte or systemic AT1R. Actually, among ARBs, the degrees of regression of cardiac hypertrophy were different between ARBs in basic research under a similarly hypertensive stimulation.12 In this study, the progression of LVH was attenuated in the TLM-treated group to a greater extent than in the CAN-treated group with similar depressor effects. However, TLM-induced sympathoinhibition occurred before the reduction of LVH. Sympathoinhibition has been considered to have prominent benefits in the treatment for heart failure and/or LVH.17 Although we did not examine cardiomyocytes or systemic AT1R, we consider that TLM has the potential to improve the survival of hypertension and heart failure with a reduction of LVH, probably due to enhanced sympathoinhibition by reduction of ROS in the RVLM.

Interestingly, survival was different between the TLM- and CAN-treated groups, not just after the initiation of each treatment. Furthermore, sympathoinhibition occurred prior to the significant difference of survival in TLM- and CAN-treated SHRSPs. We consider that these results would indicate 2 important issues. First, the improvement of survival might be associated with sympathoinhibition probably due to the blockade of AT1R in the RVLM. In this aspect, among ARBs, TLM would be a potentially reasonable agent for the treatment of hypertension and heart failure because of its blockade ability of AT1R in the RVLM. Second, long-term treatment is necessary to obtain the superior benefits of survival between ARBs. Although TLM might have the potential to be superior to other ARBs, no clinical studies have demonstrated differences in the benefits of ARBs for heart failure. In these aspects, the results in this study would have significant clinical implications.

There are several limitations in this study. First, we measured daily blood pressure by tail-cuff methods and urinary norepinephrine excretion as a parameter of cardiovascular sympathetic activity. Although it is preferable to measure blood pressure by telemetry system, as performed in our previous studies, the observation period in this study was too long for currently available radio-telemetry systems. We consider that the depressor effect in this study was almost similar between TLM- and CAN-treated SHRSPs during the experiments. Furthermore, we did not do direct sympathetic nerve recording or frequency analysis of blood pressure and/or heart rate variability. We have demonstrated that urinary norepinephrine excretion can be useful as a parameter of systemic sympathetic nerve activity.18,19,23,25 However, urinary norepinephrine excretion is not a perfect indicator of cardiovascular sympathetic activity. It is only one of the parameters of cardiovascular sympathetic activity. In future examinations, we should do continuous and telemetrically direct measuring of blood pressure, heart rate, and sympathetic nerve activity in a conscious state. Second, we used angiotensin II–infused and salt-loaded SHRSPs as a model of hypertension and heart failure. Although we determined heart failure with severe hypertension, LVH, and elevation in LVEDP, it has not been established as a specific animal model with heart failure associated with hypertension. Moreover, we did not check for death form renal function or arrhythmia, and we could not clarify whether TLM improved survival because of the benefits on heart failure or some other causes of mortality. Third, hemodynamic and echocardiographic parameters were incomplete in this study. Further studies are necessary to examine the impact of ARBs on cardiac anatomy and function in appropriate animal models of heart failure associated with hypertension used in a previous study.31 However, we believe that our animal study will provide helpful references for future clinical trials and directive information for the clinical choice among various ARBs.

In conclusion, we determined that TLM has the potential to reduce mortality, LVH, and LVEDP and that enhanced sympathoinhibition by reduction of ROS in the RVLM might be one of the mechanisms contributing to the beneficial actions of TLM in a rat model of hypertension and heart failure. Moreover, TLM might be more effective than CAN in reducing mortality, LVH, and LVEDP in hypertension and heart failure.

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DISCLOSURE

The authors declared no conflict of interest.

REFERENCES


