Effects of Telmisartan on Right Ventricular Remodeling Induced by Monocrotaline in Rats

Muneyoshi Okada1*, Toshiyuki Harada1, Ryuta Kikuzuki1, Hideyuki Yamawaki1, and Yukio Hara1

1Laboratory of Veterinary Pharmacology, School of Veterinary Medicine, Kitasato University, Towada, Aomori 034-8628, Japan

Received April 8, 2009; Accepted August 17, 2009

Abstract. The present study investigated whether telmisartan, an angiotensin II type 1 receptor antagonist, has cardioprotective effects on monocrotaline-induced right ventricular (RV) remodeling in rats. Six-week-old male Wistar rats were divided into control group (CONT), monocrotaline (60 mg/kg, i.p.)–treated group (MCT), monocrotaline (60 mg/kg, i.p.) + telmisartan (3 mg/kg per day, p.o.)–treated group (MCT+TEL), and telmisartan (3 mg/kg per day, p.o.) alone–treated group (TEL). Hearts were excised after echocardiography examinations at day 25. Significant increase in RV weight and histologically remarkable fibrosis in RV sections were observed in MCT. Tricuspid annular plane systolic excursion, a parameter for RV systolic function, significantly decreased in MCT. These RV hypertrophy, fibrosis, and dysfunction were inhibited in MCT+TEL. In MCT, the acceleration time/ejection time ratio of pulmonary artery flow velocity, an index of pulmonary hypertension, significantly decreased. This decrease was not affected in MCT+TEL. In MCT, expressions and activities of matrix metalloproteinase (MMP)-2 and MMP-9, which play a critical role in cardiac remodeling, significantly increased in the RV. In MCT+TEL, these increases in expressions and activities were inhibited. MCT showed about 2-fold increase in transforming growth factor-β1 expression compared with CONT, and such an increase was not decreased in MCT+TEL. There were no significant changes of these parameters in TEL compared with CONT. These results suggest that telmisartan could attenuate the monocrotaline-induced RV remodeling through improvements of RV hypertrophy, fibrosis, dysfunction, and inhibition of MMPs.

Keywords: angiotensin II type 1 receptor, matrix metalloproteinase, monocrotaline, right ventricular remodeling, telmisartan

Introduction

Pulmonary hypertension is a serious disease with a poor prognosis and subsequently leads to right ventricular (RV) hypertrophy and failure (1). There are various animal models for pulmonary hypertension. Single injection of monocrotaline, a pyrrolizidine alkaloid, is widely used as an idiopathic pulmonary arterial hypertension model in rats (2). Monocrotaline is metabolized to monocrotaline pyrrole in the liver. The metabolite injures pulmonary arterial endothelium and evokes pulmonary arterial hypertension. Increased pulmonary arterial pressure induces RV remodeling, including cardiac hypertrophy, fibrosis, and dysfunction (3–6).

Remodeling of the extracellular matrix (ECM), which retains a variety of signalling molecules, is critical for cell proliferation, differentiation, and cell death. Matrix metalloproteinases (MMPs), a family of zinc-dependent proteinases, regulate a part of myocardial ECM remodeling, which is fundamental to the development of cardiac diseases (7, 8). It is presumed that MMP-2 and MMP-9, which belong to the gelatinase group of MMPs, promote cardiac remodeling in various cardiac diseases, including myocardial infarction, left ventricular (LV) hypertrophy, and cardiomyopathy (9). In monocrotaline-treated RV myocardium of rats, MMP-2 and MMP-9 were increased and might have an important role in RV remodeling (10).
Several studies reported that the renin-angiotensin system (RAS) is activated in monocrotaline-induced hypertrophied RV (11, 12). We previously reported that an angiotensin-converting enzyme (ACE) inhibitor, captopril, attenuates monocrotaline-induced RV hypertrophy, fibrosis, dysfunction, and MMP-2 and MMP-9 expressions in rats (13). Therefore, it is assumed that RAS promotes the development of monocrotaline-induced RV remodeling. The effects of angiotensin II type 1 receptor (AT1R) blockers in monocrotaline-induced RV hypertrophy have not yet been completely clarified. Losartan did not inhibit monocrotaline-induced RV hypertrophy (14), while candesartan prevented it (15). Telmisartan, a 5th generation AT1R blocker, is used for the treatment of hypertension and has cardioprotective effects (16). However, little is known about the effects of telmisartan on RV hypertrophy. The aim of the present study is to investigate the influence of telmisartan on monocrotaline-induced RV remodeling, by focusing on cardiac hypertrophy, fibrosis, dysfunction, and MMPs.

Materials and Methods

Animal models

All animals were cared for in accordance with the guidelines for animal treatment of Kitasato University, which meet international guiding principles of laboratory animal care. Six-week-old male Wistar rats (Clea Japan, Inc., Tokyo) were maintained on a standard laboratory diet and tap water, and exposed to a 12 h/12 h light-dark cycle at 23 ± 2°C throughout experiments. Rats were divided into 4 groups: control group (CONT), monocrotaline-treated group (MCT), monocrotaline + telmisartan–treated group (MCT+TEL), and telmisartan alone–treated group (TEL). MCT and MCT+TEL were injected with monocrotaline (60 mg/kg, i.p.; Wako Pure Chemical Industries, Ltd., Osaka), which was dissolved in 1 N HCl and neutralized with 1 N NaOH. CONT and TEL were injected with saline (2.5 ml/kg, i.p.). Telmisartan (Micardis®; Boehringer Ingelheim, Ingelheim, Germany) was suspended in distilled water and administered (3 mg/kg per day, p.o.) for 24 days from the day of monocrotaline-injection to MCT+TEL and TEL. CONT and MCT were administered with distilled water.

Echocardiography

Echocardiography was performed at day 25 after monocrotaline-injection under pentobarbital (50 mg/kg, i.p.) anesthesia using SONOS 5500 (Hewlett-Packard Co., Andover, MA, USA) with a dynamically focused S12 probe (5–12 MHz, Hewlett Packard Co.) as described previously (13). Acceleration time (AT) and ejection time (ET) were measured from pulsed Doppler of pulmonary artery flow velocity. Tricuspid annular plane systolic excursion (TAPSE), the motion of tricuspid annulus, was measured in the M-mode from the apical four chamber view. AT/ET ratio and TAPSE were used as an index of pulmonary hypertension (17, 18) and a parameter for RV systolic function (19, 20), respectively. We confirmed that pentobarbital (50 mg/kg, i.p.) did not cause a significant respiratory depression, which would influence the AT/ET ratio and TAPSE.

Histology

After echocardiographic examination, hearts were excised for histological and biochemical examinations. The hearts were separated into right and left atrial or ventricular tissues. Isolated ventricular tissues and lungs were weighed. The ventricular tissues for histological examinations were fixed in 10% neutral buffered formalin for histological examination. Thin paraffin sections (2 μm) were made and Azan staining was performed by a standard procedure (13).

Western blot analysis

Western blot analysis was performed as described previously (13). Briefly, RV samples were homogenized in lysis buffer [20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA-2Na, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, 1 mg/ml leupeptin; Cell Signaling Technology, Inc., Danvers, MA, USA] containing 1% protease inhibitor cocktail (Nacalai Tesque, Inc., Kyoto) and centrifuged. The supernatant was used for Western blot analysis. The proteins [20 μg for MMP-9 and transforming growth factor (TGF)-β1; 60 μg for MMP-2] were separated on 7.5% (for MMP2 and MMP-9) and/or 12% (for TGF-β1) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. After blocking with 0.5% skim milk, the membranes were incubated with rabbit polyclonal antibodies against MMP-2 (Thermo Fisher Scientific Anatomical Pathology, Fremont, CA, USA), MMP-9 (Millipore Co., Billerica, MA, USA), TGF-β1 (Biovision Research Products, Mountain View, CA, USA), and mouse monoclonal antibody against total actin (Sigma-Aldrich Co., St, Louis, MO, USA). HRP-conjugated anti-rabbit IgG or anti-mouse IgG was used for the secondary antibody. Signal detection was archived using the ECL plus western blotting detection reagents (GE Healthcare Ltd., Buckinghamshire, UK) in the ATTO light capture system (AE-6972; ATTO Co., Tokyo).
Gelatin zymography

RV proteins (10 μg) were separated on 7.5% SDS-PAGE containing 1.8 mg/ml gelatin under non-reducing conditions. After incubating for 1 h in washing buffer [50 mM Tris-HCl (pH 7.5), 2.5% Triton X-100, 5 mM CaCl$_2$, 1 μM ZnCl$_2$], gels were incubated overnight in incubation buffer [50 mM Tris-HCl (pH 7.5), 5 mM CaCl$_2$, 1 μM ZnCl$_2$] at 37°C. Then the gels were stained with 0.1% Coomassie Blue G-250 for 20 min and destained with ion-exchanged water until bands were visible.

Statistical analyses

The results are presented as means ± S.E.M. Statistical analyses were performed by one-way ANOVA followed by Tukey’s post-hoc test. A value of $P<0.05$ was considered to be statistically significant.

Results

**Telmisartan attenuated monocrotaline-induced RV hypertrophy**

Table 1 shows the biometrical changes of rats at day 25 after monocrotaline injection. Body weight (BW) of MCT significantly decreased compared with CONT ($P<0.01$), and this decrease was not prevented in MCT+TEL. As tail length (TL) did not change among the 4 groups, RV weight (RVW), LV + interventricular septum weight (LV+IVSW), and lung wet weight (LWW) were corrected by TL. In MCT, RVW/TL ratio significantly increased compared with CONT ($P<0.01$). In MCT+TEL, RVW/TL ratio significantly decreased compared with that in MCT ($P<0.01$). LV+IVSW/TL ratio was not different among the 4 groups. LWW/TL ratio in MCT significantly increased compared with CONT ($P<0.01$), and this increase was not prevented in MCT+TEL. All the parameters except for BW in TEL were not changed compared with CONT.

**Telmisartan suppressed monocrotaline-induced fibrosis in RV**

Fibrosis was assessed by Azan staining in RV sections (Fig. 1). In MCT, remarkable fibrosis was observed in RV sections (Fig. 1B, arrowhead). In the RV section of MCT+TEL, fibrosis was significantly attenuated (Fig. 1C). No histological abnormality was observed in CONT and TEL (Fig. 1: A and D). Reappearance of the same histological changes was confirmed in different heart preparations (CONT, n = 3; MCT, n = 6; MCT+TEL, n = 5; TEL, n = 4).

![Fig. 1. Effects of telmisartan on monocrotaline-induced fibrosis in rat right ventricle (RV). Representative Azan staining sections from RV of the control (A), monocrotaline-treated (B), monocrotaline + telmisartan–treated (C), and telmisartan alone–treated (D) groups are shown. Remarkable fibrosis was shown in monocrotaline-treated RV (B, arrowhead). Scale bars represent 100 μm.](image-url)

<table>
<thead>
<tr>
<th></th>
<th>CONT (n = 10)</th>
<th>MCT (n = 13)</th>
<th>MCT+TEL (n = 16)</th>
<th>TEL (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>303 ± 6</td>
<td>251 ± 5**</td>
<td>259 ± 3**</td>
<td>283 ± 4*††</td>
</tr>
<tr>
<td>TL (cm)</td>
<td>15.9 ± 0.2</td>
<td>15.8 ± 0.1</td>
<td>15.4 ± 0.1</td>
<td>15.8 ± 0.1</td>
</tr>
<tr>
<td>RVW (mg)</td>
<td>168 ± 6</td>
<td>356 ± 17**</td>
<td>242 ± 17**††</td>
<td>143 ± 7†</td>
</tr>
<tr>
<td>LV+IVSW (mg)</td>
<td>655 ± 16</td>
<td>619 ± 18</td>
<td>583 ± 10**</td>
<td>599 ± 19</td>
</tr>
<tr>
<td>LWW (mg)</td>
<td>1221 ± 30</td>
<td>2202 ± 102**</td>
<td>1899 ± 87**††</td>
<td>1151 ± 17††</td>
</tr>
<tr>
<td>RVW/TL ratio (mg/cm)</td>
<td>10.6 ± 0.4</td>
<td>22.6 ± 1.2**</td>
<td>15.7 ± 1.1**††</td>
<td>9.1 ± 0.4†</td>
</tr>
<tr>
<td>LV+IVSW / TL ratio (mg/cm)</td>
<td>41.2 ± 1.1</td>
<td>39.4 ± 1.2</td>
<td>37.9 ± 0.7</td>
<td>38.1 ± 1.2</td>
</tr>
<tr>
<td>LWW/TL ratio (mg/cm)</td>
<td>76.9 ± 2.5</td>
<td>140.0 ± 7.0**</td>
<td>123.4 ± 5.8**</td>
<td>73.1 ± 1.3††</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.E.M. *$P<0.05$, **$P<0.01$: compared with CONT. †$P<0.05$, ††$P<0.01$: compared with MCT. CONT: control group, MCT: monocrotaline-treated group, MCT+TEL: monocrotaline + telmisartan–treated group, TEL: telmisartan alone–treated group. BW: body weight, TL: tail length, RVW: right ventricular weight, LV: left ventricular, IVSW: interventricular septum weight, LWW: lung wet weight.
Effects of telmisartan on AT/ET ratio and TAPSE

The effects of telmisartan on pulmonary hypertension and RV systolic function were evaluated by measuring AT/ET ratio and TAPSE, respectively, in echocardiography. AT/ET ratio in MCT significantly decreased compared with CONT ($P < 0.01$, Table 2), and this decrease was not prevented in MCT+TEL. In MCT, TAPSE significantly decreased compared with CONT ($P < 0.01$, Table 2). In MCT+TEL, this parameter significantly increased compared with MCT ($P < 0.01$). AT/ET ratio and TAPSE in TEL were not changed compared with CONT.

Telmisartan attenuated monocrotaline-induced MMP-2 and MMP-9 expressions and activities in RV

To investigate the effects of telmisartan on MMP-2 and MMP-9 expressions in RV, we used Western blotting analysis (Fig. 3: A – D). Both MMP-2 and MMP-9 expressions increased significantly in MCT (208 ± 19% and 236 ± 25%, respectively, n = 13, $P < 0.01$) compared with CONT (n = 10). In MCT+TEL, MMP-2 and MMP-9 expressions significantly decreased compared with MCT (137 ± 18% and 161 ± 19%, respectively, n = 16, $P < 0.05$). Telmisartan alone–treatment had no effect on MMP-2 and MMP-9 expressions (119 ± 15% and 110 ± 17%, respectively, n = 10) compared with CONT. Gelatin zymography was performed to evaluate the effects of telmisartan on MMP-2 and MMP-9 activities in RV (Fig. 4: A – C). Both MMP-2 and MMP-9 activities increased significantly in MCT (135 ± 4% and 239 ± 22%, respectively, n = 13, $P < 0.01$) compared with CONT (n = 10). In MCT+TEL, these activities significantly inhibited (113 ± 4%, $P < 0.01$ and 175 ± 19%, $P < 0.05$, respectively, n = 16). Telmisartan alone–treatment had no effect on MMP-2 and MMP-9 activities (95 ± 3% and 111 ± 11%, respectively, n = 10) compared with CONT.

Telmisartan did not attenuate monocrotaline-induced TGF-β1 expression in RV

We finally investigated the effects of telmisartan on TGF-β1 expression in RV (Fig. 5: A and B). In MCT, TGF-β1 expression increased by about 2-fold (193 ± 40%, n = 13) compared with CONT (n = 10), but not significantly. In MCT+TEL, TGF-β1 expression did not decrease (207 ± 31%, n = 16) compared with MCT. Telmisartan alone–treatment had no effect on TGF-β1 expression (118 ± 18%, n = 10) compared with CONT.

Discussion

In the present study, we revealed that telmisartan
Effects of Telmisartan on RV Remodeling 197

attenuates monocrotaline-induced RV hypertrophy, fibrosis, dysfunction, and the increased expressions and activities of MMP-2 and MMP-9. To the best of our knowledge, this is the first report about the inhibitory effects of telmisartan on monocrotaline-induced RV remodeling.

The monocrotaline-induced RV hypertrophy model in rats is well known as the animal model for idiopathic pulmonary arterial hypertension (2). In this report, we produced monocrotaline-induced RV hypertrophy by injecting monocrotaline (60 mg/kg, i.p.) into rats. RVW/TL ratio of MCT significantly increased compared with CONT and remarkable fibrosis was shown in RV sections of MCT. These features of MCT

Fig. 3. Effects of telmisartan on monocrotaline-induced MMP-2 and MMP-9 expressions in right ventricles (RV) of the control group (CONT), monocrotaline-treated group (MCT), monocrotaline + telmisartan–treated group (MCT + TEL), and telmisartan alone–treated group (TEL). Representative blots for MMP-2 (C) and MMP-9 (A) are shown. Equal loading of protein was confirmed with anti-actin antibody. Levels of MMP-2 (D) and MMP-9 (B) expressions relative to CONT are shown as means ± S.E.M. (CONT, n = 10; MCT, n = 13; MCT + TEL, n = 16; TEL, n = 10). **P < 0.01: compared with CONT.

Fig. 4. Effects of telmisartan on monocrotaline-induced MMP-2 and MMP-9 activities in right ventricles (RV) of the control group (CONT), monocrotaline-treated group (MCT), monocrotaline + telmisartan–treated group (MCT + TEL), and telmisartan alone–treated group (TEL). Representative zymograms for MMP-2 and MMP-9 are shown in panel A. Levels of MMP-2 (C) and MMP-9 (B) activities relative to the CONT group are shown as means ± S.E.M. (CONT, n = 10; MCT, n = 13; MCT + TEL, n = 16; TEL, n = 10). *P < 0.05, **P < 0.01: compared with CONT. †P < 0.05, ††P < 0.01: compared with MCT.
corresponded to the previous reports (3–6). It was reported that AT1R blockers, such as olmesartan medoxomil (5 mg/kg per day) (21) or candesartan (10 mg/kg per day) (15), prevent RV hypertrophy induced by chronic hypoxia or monocrotaline, respectively. Consistent with these reports, the present study demonstrated that telmisartan significantly decreased RVW/TL and RV fibrosis in MCT. Several studies have indicated that TAPSE, an index of RV function by echocardiography, was significantly decreased by monocrotaline treatment in rats (19, 20). In the current study, we observed significant decrease of TAPSE in MCT, and significant recovery in MCT + TEL. Thus, it is suggested that telmisartan ameliorates RV dysfunction induced by monocrotaline-treatment.

MMP-2 and MMP-9, which degrade ECM components, play a critical role in the cardiac remodeling process (9). In the present study, MMP-2 and MMP-9 expressions and activities were significantly increased in MCT, which corresponded to our recent report (13). Although it is presumed that RAS is responsible for the induction of MMPs in cardiac remodeling (22), the precise effect of angiotensin II has not been clarified. Takenaka et al. reported that telmisartan (5 mg/kg per day) significantly decreased MMP-2 and MMP-9 mRNA expressions in the congestive heart failure model in Dahl salt-sensitive rats (23). We have recently reported that an ACE inhibitor, captopril, attenuated MMP-2 and MMP-9 expressions in RV induced by monocrotaline-treatment (13). In the present study, telmisartan-treatment significantly decreased MMP-2 and MMP-9 expressions and activities in MCT. Therefore it is suggested that angiotensin II, perhaps in part, induces MMP-2 and MMP-9 expressions during the development of RV remodeling through AT1R. The activities of MMPs are specifically regulated by tissue inhibitor of metalloproteinases (TIMPs), and altered balance of MMPs and TIMPs promotes cardiac ECM remodeling (7, 8). To elucidate the role of TIMP-1 in the regulation of MMPs activities, we examined TIMP-1 expressions in monocrotaline-treated RV. In the present study, however, TIMP-1 expression in MCT did not change compared with CONT or MCT + TEL (data not shown). Therefore, it is suggested that TIMP-1 might not have an important role in the deactivation of MMP-2 and MMP-9 activities in monocrotaline-treated RV.

It has been proposed that angiotensin II upregulates TGF-β1 expression via activation of AT1R in cardiac myocytes and fibroblasts and that increased TGF-β1 promotes cardiac remodeling (24). Therefore, telmisartan-treatment was expected to suppress monocrotaline-induced RV remodeling through the regulation of TGF-β1 expression. In the present study, TGF-β1 expression in MCT increased by about 2-fold compared with CONT. However, telmisartan did not suppress this increase. From these results, it is suggested that telmisartan might not attenuate monocrotaline-induced RV remodeling through the inhibition of TGF-β1 expression in rats.

Several studies have reported that the AT and AT/ET ratio, which were used as an index of pulmonary hypertension, significantly decreased in monocrotaline-treated rats (17, 18). Consistent with these reports, AT/ET ratio in MCT significantly decreased in our experiment. It is controversial whether AT1R blockers attenuate pulmonary hypertension. Losartan (10 mg/kg per day) did not inhibit monocrotaline-induced pulmonary hypertension and RV hypertrophy (14). On the other hands, Kishi et al. reported that candesartan (10 mg/kg per day) prevented the decrease of AT, the increase of pulmonary arterial pressure, and RV hypertrophy induced by monocrotaline treatment (15). The present study demonstrated that telmisartan did not improve the decrease of AT/ET ratio, but attenuated RV hypertrophy. Therefore, telmisartan might attenuate RV hypertrophy without suppressing pulmonary hypertension. There are several reasons for this inconsistency in the effects of these AT1R antagonists on monocrotaline-induced pulmonary hypertension and RV hypertrophy in rats. First, losartan has the shortest plasma half-life and the weakest AT1R binding affinity among other
sartans, including candesartan and telmisartan (25, 26). Therefore losartan could not inhibit pulmonary hypertension and RV hypertrophy induced by monocrotaline treatment in rats. Second, candesartan but not telmisartan improved monocrotaline-induced pulmonary hypertension in rats. The reason for this may be due to differences in dosage and in AT1R binding affinity between candesartan and telmisartan. Although candesartan was given at 10 mg/kg per day (15), we used telmisartan at 3 mg/kg per day in the present study. Moreover, the AT1 binding affinity of candesartan is greater than that of telmisartan (26). Therefore, it is speculated that treatment with telmisartan at 3 mg/kg per day was not enough to improve pulmonary artery hypertension. Third, Zou et al. demonstrated that activation of AT1R induced by mechanical stress was inhibited by the inverse agonistic activity of candesartan (27). It is possible that AT1R antagonists with inverse agonistic activity could effectively inhibit pulmonary hypertension and RV hypertrophy induced by monocrotaline treatment. However, losartan, which is also considered to have an inverse agonistic activity (28), failed to inhibit monocrotaline-induced pulmonary hypertension and RV hypertrophy. Therefore it seems unlikely that AT1-receptor antagonists with inverse agonistic activity can always inhibit RV hypertrophy associated with pulmonary hypertension. In addition, it is known that telmisartan acts as a partial agonist for peroxisome proliferators-activated receptor-γ (PPARγ) (29). This function is specific to telmisartan among several AT1R antagonists, including candesartan and losartan (30). Several reports suggested that PPARγ agonists have beneficial effects on cardiovascular diseases (31–33). Nakamura et al. reported that combination of candesartan and pioglitazone, a PPARγ agonist, more significantly attenuated LV hypertrophy than treatment with candesartan alone in stroke-prone spontaneously hypertensive rats (32). Kobayashi et al. reported that the cardioprotective mechanism of telmisartan is partly due to activation of PPARγ-dependent endothelial nitric oxide synthase pathway in Dahl salt-sensitive hypertensive rats (33). Therefore, it can be postulated that both a blocking action on AT1R and an agonistic action for PPARγ are related to suppressive effects of telmisartan on RV hypertrophy induced by monocrotaline. Further studies are needed to clarify whether the effects of telmisartan on RV hypertrophy are mediated through AT1R antagonism or/and activation of PPARγ.

In summary, telmisartan suppressed monocrotaline-induced RV hypertrophy, fibrosis, and dysfunction and inhibited the elevation of MMP-2 and MMP-9 in monocrotaline-treated rats. These beneficial effects of telmisartan on monocrotaline-induced RV remodeling appear to occur in the absence of an effect on pulmonary artery pressure and, probably, in the absence of an effect on lung pathology.

Acknowledgment

This research was supported in part by Grant for Encouragement of Young Scientists to M. Okada from School of Veterinary Medicine, Kitasato University.

References


Effects of Telmisartan on RV Remodeling


