Effects of masitinib compared with tadalafil for the treatment of monocrotaline-induced pulmonary arterial hypertension in rats

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Abstract

Targeting vascular remodeling in pulmonary arterial hypertension (PAH) remains a challenge given the lack of potent anti-remodeling abilities of the therapeutic drugs. Although sildenafil has been shown to ameliorate cardiopulmonary remodeling, that of tadalafil is questionable. Masitinib, a tyrosine kinase inhibitor appears safer and more potent than imatinib for treatment of malignancies, but its efficacy on PAH is unknown. Therefore, we investigated the anti-remodeling properties of masitinib (5, 15, 50 mg/kg) and tadalafil (5, 10 mg/kg) using a monocrotaline-induced rat model of PAH. The 14-day treatment with masitinib (15, 50 mg/kg) resulted in significantly decreased right ventricular (RV) systolic pressure (RVSP) and hypertrophy (RVH), and pulmonary vascular remodeling, whereas tadalafil showed weaker anti-remodeling properties. Besides, masitinib significantly blocked the mitogen-associated protein kinase (MAPK) pathway, and reduced phosphodiesterase (PDE)-5 mRNA expression in the lungs. By contrast, tadalafil did not significantly inhibit the MAPK pathway. Further, the 28-day treatment extension revealed that masitinib-treated rats (15 mg/kg) had significantly lower RVSP, and higher heart rate and serum cyclic guanosine monophosphate (cGMP) level, whereas those treated with tadalafil (10 mg/kg) showed insignificantly lower RVSP and higher cGMP level. Moreover, the RVH indices, heart rates, body weight gains, and survival rates of rats in both groups were comparable. Collectively, these results suggest that the treatment with a low-dose masitinib was non-inferior than tadalafil. A lower dose of masitinib may represent a novel approach to target both the cardiopulmonary remodeling and the dysregulated vasoconstriction in PAH.

Keywords: Cardiopulmonary remodeling, masitinib, pulmonary arterial hypertension, right ventricular hypertrophy, tadalafil

1. Introduction

Pulmonary arterial hypertension (PAH) comprises remodeling [28] and imbalance of constriction-dilation tonus [7] of the pulmonary vasculature. In an effort to improve response rates and prolong survival times in the PAH patients, combination therapy has emerged as an alternative to the conventional vasodilator monotherapy [20] for patients newly diagnosed with classic forms of PAH [33]. Nevertheless, the lack of a potent anti-remodeling ability of endothelin receptor antagonists, phosphodiesterase-5 inhibitors, guanylate cyclase stimulators, and prostacyclin derivatives which constitute the current treatment algorithm [33] remains an inconvenient truth.

Vascular remodeling, an important aspect of the PAH pathobiology, is driven by a dysregulated signaling from various pathways [33], particularly the mitogen-activated protein kinase (MAPK) pathway [5]. With this respect, we demonstrated that lower doses of imatinib [24] and sorafenib [22] inhibited the MAPK pathway and ameliorated pulmonary vascular remodeling and right ventricular (RV) hypertrophy in the MCT-injected rats. Hemodynamically, the low-dose sorafenib also decreased the RV systolic pressure (RVSP) in the rats [22], whereas the low-dose imatinib improved the RV hemodynamics in humans [15] and dogs [4, 23].

Masitinib is a veterinary drug approved for the treatment of canine mast cell tumors [27]. Compared with imatinib, it has a higher affinity for wild-type KIT proto-oncogene receptor tyrosine kinase (C-KIT) (IC_{50} = 0.15 μM, versus 0.41 μM for imatinib) and platelet-derived growth factor receptor beta (PDGFR-β) (IC_{50} = 0.02 μM, versus 0.44 μM for imatinib), but lacks activity against cardiotoxic breakpoint cluster region-abelson (Bcr-Abl) kinase (IC_{50} = 1.5 μM, versus 0.2 μM for imatinib) [34]. While the similar kinase profiles of masitinib with imatinib may raise a concern of

safety as what was observed in the IMPRES extension study of imatinib [12], increasing evidence suggests that masitinib is not only more potent but also safer than imatinib [2,6,21,25]. Further, masitinib at a dose of 50 mg/day/head improved RV contractility and reduced pulmonary medial hypertrophy in monocrotaline (MCT)-injected rats [19], thus providing a compelling rationale to investigate the potential use of a low-dose masitinib for the treatment of PAH. We hypothesized that the low-dose masitinib therapy may be effective for the treatment of PAH and cardiovascular remodeling while avoiding adverse effects of a neoplastic therapeutic dose.

Tadalafil, a phosphodiesterase (PDE)-5 inhibitor, prevents hydrolysis of cyclic guanosine monophosphate (cGMP) to 5'-GMP and causes pulmonary artery vasodilation [19]. As an FDA-approved drug for the PAH treatment [9], the beneficial effects of tadalafil on PAH hemodynamics have been extensively reported [3,13,14,31]. Recent studies have also shown that sildenafil, another PDE-5 inhibitor attenuates cardiopulmonary remodeling by antagonizing C-KIT$^+$ cells, CXC chemokine receptor (CXCR) 4 [11], and the MAPK pathway [18]. Collectively, these findings suggest that tadalafil may also potentially prevent the cardiopulmonary remodeling, thus eliciting greater treatment outcomes than the tyrosine kinase inhibitors via dual inhibition of the PDE-5 and the MAPK pathway.

Given the unknown effect of a low-dose masitinib for PAH, the present study used three doses (5, 15, and 50 mg/kg) to determine the optimal dose that would significantly prevent pulmonary arterial remodeling and RV hypertrophy in the MCT-injected rats after 14 days of treatment. Next, in the extension study to compare the non-inferiority of the two different drugs, the masitinib dose was selected based on the lowest dose that exhibited statistically significant anti-remodeling properties: the rats

were treated with masitinib (15 mg/kg) and the clinical dose of tadalafil (10 mg/kg). The outcomes and survivals of the rats in both groups were examined after 28 days of treatments.

2. Methods

2.1. Monocrotaline-induced PAH and treatments

The use of male Wistar-Imamichi rats in the study was in accordance to the Institutional Animal Care and Use Committee of the Tottori University, as described previously [22,24]. To induce PAH, the placebo and treatment rats were injected with monocrotaline (MCT, 60 mg/kg, Sigma-Aldrich, China), subcutaneously. Rats in the control group received physiologic saline. Fourteen days after injection, the treatment rats were medicated with masitinib [Masivet 50 mg, AB Science, France: 5 (masi-5), 15 (masi-15), or 50 (masi-50) mg/kg per day] or tadalafil [Tadacip 20 mg, Cipla Ltd, India: 5 (tada-5) or 10 (tada-10) mg/kg per day]. Rats in the control and MCT groups were given water. The treatments were administered orally, once daily, for 14 days. To compare the long-term outcomes between the masi-15 and tada-10 groups, the treatments were extended for an additional 14 days to reach the end point.

2.2. Hemodynamic measurements

To measure the right ventricular systolic pressure (RVSP) and heart rate (HR), a polyethene catheter (PE-50 Intramedic PE tubing, Becton Dickinson) was inserted into the right jugular vein and advanced to the RV of the isoflurane-anesthetized rats. The isoflurane was maintained at 1-2% during the cannulation. The data were recorded and analyzed with the PowerLab System connected to a pressure transducer (ADInstruments).

2.3. Assessment of RVH

The RV tissue was separated from the left ventricle and septum (LV+S). Wet weights of the RV and LV+S were used to obtain the RVH index, calculated by [RV/(LV+S)]. Further, the [RV/(LV+S)] ratio was divided by body weight (g) to obtain RVH normalized to body weight values.

2.4. Assessment of pulmonary arterial remodeling

Pulmonary arterial muscularization was assessed from the left lung lobes stained with elastic van Gieson. The number of small pulmonary arteries (20–50 μm) that were fully (FMPA), partially (PMPA), and non-muscularized (NMPA) were counted, as described previously [22,24]. Using Image J software, the FMPA that were 20–50 μm and 51–100 μm in diameter were examined further to determine the external diameter (*d*), medial wall thickness (MWT), MWT ratio (2 × MWT / *d* × 100%), lumen diameter (*d*–2 × MWT), and lumen area [3.142 × (*d*/2)²].

2.5. Fast real-time polymerase chain reaction

The expression levels of the target messenger RNAs (mRNAs) in the RV tissues and the right caudal lung lobes were determined using Applied Biosystems 7500 Fast Real-Time PCR System with TB Green Fast qPCR Mix (Takara Bio Inc., Shiga, Japan). In addition to the previously described mRNAs [22,24], phosphodiesterase (PDE)-5 (forward: TCCCCGGTTCAATGCAGAAG; reverse: GATGGCCTGAGCTACACCAA) expression levels were also determined.

2.6. Western blotting assay

The procedures were carried out as described previously [22,24]. Lysates containing 50 μg total protein from the right caudal lung lobes were electrophoresed on 4–15% sodium dodecyl sulfate-polyacrylamide gels (Mini-PROTEAN® TGX™ Precast

Protein Gels, Bio-Rad, USA) and transferred to polyvinylidene difluoride membranes. The membranes were blocked with Tris-buffered saline (EzTBS, Atto, Japan) containing 1% bovine serum albumin for 1 hour at room temperature, and then incubated with anti-phospho-extracellular-signal-related kinase (ERK) 1/2 (Cell Signaling Technology, Inc., USA), anti-ERK 1/2 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), anti-PDGF B (Abcam, United Kingdom), and anti-β-actin (Abcam, United Kingdom) for 4-5 hours at room temperature, followed by a specific secondary antibody. The antibody-antigen complexes were visualized using Bio-Rad Universal Hood II whereas the protein expression was quantified using Image Lab Software 6.0 (Bio-Rad Laboratories).

2.7. ELISA measurement of serum cyclic guanosine monophosphate (cGMP)

Intra-cardiac puncture was performed to collect blood into plain blood tubes. Serum was obtained by centrifuging the clotted blood at 3,500 rpm for 5 minutes (Kubota 4000, Japan). Enzyme-linked immunosorbesnt assay (ELISA) for rat serum cGMP was carried out using a commercial kit (Abcam, United Kingdom), in accordance to the manufacturer's protocol. Absorbance was read at 450 nm by an IMark™ microplate reader (Bio-Rad Laboratories, Japan) and a standard curve was constructed to give readings of the cGMP (ρmol).

2.8. Statistical analyses

To confirm the establishment of PAH, differences between the MCT-injected and control groups were analyzed using Student's t-test for normally distributed data or Mann–Whitney U test for non-normally distributed data. Differences between the treatment groups and MCT-injected group were determined by one-way analysis of variance followed by Dunnett's post-hoc test, or Mann–Whitney U test. Scatter plots

were constructed to observe linearity and the dose-dependent relationship was analyzed by Pearson correlation test. Survival curve was constructed using Kaplan-Meier method and analyzed by Log-Rank test. Data are presented as means ± standard error of the mean (SEM) and considered statistically significant at $P < 0.05$.

3. Results

3.1 Masitinib versus tadalafil treatments on the RVSP and heart rate (HR)

The PAH development was indicated by a significantly increased RVSP in the MCT-injected rats, compared with that in the control rats (Fig. 1a). In the treatment groups, the masi-15, masi-50, tada-5, and tada-10 rats showed lower RVSP than the MCT-injected group. The reduction was dose-dependent in the masitinib treatment (Fig. 1b). All groups had comparable HRs (Fig. 1a).

3.2. Masitinib versus tadalafil treatments on the RVH

With respect to the cardiac remodeling, we observed a dose-dependent decrease in RVH by the masitinib treatment (Fig. 1c). While the MCT-injected rats showed severe RV enlargement indicated by an increased RVH index, compared with than that of the control (Fig. 1d), the masi-15, masi-50, and tada-10 groups significantly decreased the RVH, compared with the MCT group. When the RVH was normalized to body weight, only the masi-15 and masi-50 treatments exhibited a significantly lower RVH index than the MCT rats (Fig. 1d).

3.3. Masitinib versus tadalafil treatments on the BNP mRNA expression of the RV

The above findings were further supported by the BNP mRNA expression of the RV (Fig. 1e). The mRNA expression was significantly upregulated in the MCT-injected rats compared with the control. Masitinib dose-dependently decreased the mRNA levels (Fig. 1f), and those of the masi-15 and masi-50 rats were significantly lower than that of the MCT-injected rats. By contrast, in the tadalafil groups, the tada-5 and tada-10 treatments did not significantly reduce the mRNA levels.

3.4. Masitinib versus tadalafil treatments on remodeling in 20-50 μm pulmonary arteries

3.4.1. Degree of muscularization

The different types of distal PAs are shown in Fig 2a. We showed that the control rats had the highest percentage NMPA, whereas the MCT group had fewer thin-walled PAs by comparison (Fig. 2b). Compared with the MCT group, the masitinib therapy increased the NMPA percentage in a dose-dependent manner (Fig. 2c). By contrast, neither the tada-5 nor tada-10 significantly increased the NMPA percentage.

Further, the MCT-injected rats also showed higher FMPA percentage than the control (Fig. 2b), indicating PA muscularization. We also observed occlusive lesions in the FMPAs (Fig. 2a [v, vi]), implying a marked appearance of remodeled PAs after 28 days of the MCT treatment. The FMPA percentage was significantly and dose-dependently decreased in the masitinib groups (Fig. 2d). Although the reduction was also significant in the tadalafil-treated rats, the FMPA percentages were much higher than that of the masitinib-treated rats.

3.4.2. Medial wall thickness (MWT) ratio and lumen area

Next, we examined MWT and lumen area of the PAs as indicators of pulmonary remodeling. Compared with the control, the detailed analysis of the FMPA (Fig. 3a) showed noticeable medial hypertrophy in the MCT group, indicated by an increased MWT ratio. In the treatment groups, a significant MWT reduction was observed in the masi-15, masi-50, tada-5, and tada-10 groups.

Likewise, the lumen area of the FMPA in the MCT group (Fig. 3b) was also decreased, compared with that in the control group. Except the masi-5 group, all treatment groups significantly increased the lumen area. Nonetheless, those of the tadalafil-treated rats were smaller than those of the masi-15 and masi-50 rats.

3.5. Masitinib versus tadalafil treatments on remodeling in 51─100 μm pulmonary arteries

In the larger pulmonary arteries, the MCT group also showed increased MWT ratio and reduced lumen area, compared with those in the control (Fig. 3c, d). Although insignificant, the masi-15, masi-50, and tada-10 groups exhibited a much lower MWT ratio than the MCT group. Further, the masi-15, masi-50, and tada-10 treatments also significantly increased the lumen area.

3.6. Masitinib versus tadalafil treatments on the expression of receptors C-KIT and *PDGFR-β mRNAs and PDGF-B protein in the lungs*

Furthermore, we examined the expression of various tyrosine kinase receptors in the rat lungs. Compared with those of the control rats, the rat lungs in the MCT group exhibited increased levels of C-KIT (Fig. 4a) and PDGFR-β (Fig. 4b) mRNAs, as well as PDGF-B protein (Fig. 4c, d). All doses of masitinib reduced the C-KIT and tended to inhibit the PDGFR-β mRNA levels. We also observed a significant decrease in the PDGF-B protein level in the masi-50 group. The tadalafil-treated rats also tended to reduce the C-KIT and PDGFR-β mRNAs, and PDGF-B protein.

3.7. Masitinib versus tadalafil treatments on the pulmonary CXCR4 / CXCL12 axis

The CXCR4 mRNA expression in the lung tissues of the MCT-injected rats was significantly upregulated, compared with the control (Fig. 5a). In the treatment groups, only the masi-15 and masi-50 treatments suppressed the CXCR4 mRNA expression, whereas no significant inhibition was observed in the masi-5, tada-5, and tada-10 groups.

Besides, the CXCL12 mRNA levels were significantly higher in the MCT-injected lungs, compared with the control (Fig. 5b). While the masi-15 and masi-50 treatments reduced the mRNA expression by 3.0 and 2.5 folds, respectively, the tadalafil groups showed comparable mRNA levels with the MCT group.

3.8. Masitinib versus tadalafil treatments on the MAPK signaling pathway

Given the upregulation of tyrosine kinase receptor and CXCR4/CXCL12 mRNAs and PDGF-B protein, Raf-1 mRNA as an entry point to the MAPK pathway was also upregulated in the MCT group (Fig. 5c). Among the treatment groups, only the masi-15 and masi-50 treatment groups showed significantly lower mRNA level, compared with the MCT group.

In tandem with the above results, the MCT-injected lungs exhibited a higher phosphorylated ERK protein level than the control (Fig. 5 d, e), confirming the role of MAPK signaling in the development of PAH. As expected, the masi-15 and masi-50 groups showed significantly reduced phosphorylated ERK protein level, which was lacking in the tadalafil treatment groups.

3.9. Masitinib versus tadalafil treatments on the PDE-5 and cGMP levels

After 28 days of the MCT injection, the MCT rats showed a higher PDE-5 mRNA level than the control (Fig. 6a). Compared with that of the MCT rats, all the treatment rats showed reduced mRNA levels, and the decrease was significant in the masi-50 and tada-5 groups.

After 42 days of the MCT injection, serum cGMP concentration was reduced in the MCT rats compared with that of the control (Fig. 6b). While the tada-10 treatment tended to increase the serum level, the masi-15 rats yielded a higher serum cGMP concentration than the MCT rats.

3.10. Masitinib 15 mg/kg versus tadalafil 10 mg/kg treatments on the long-term outcomes in the rats

Given the beneficial effects of the masi-15 treatment as shown above, we further evaluated the long-term outcomes in the rats treated with masi-15 versus tada-10. In the MCT group, death occurred as early as 22 days after the MCT-injection (Fig. 7a), and eight of 10 (80%) rats were alive at the end point on day 40. In the masi-15 group, deaths occurred on days 35 and 39 post MCT-injection, and seven of 9 (78%) rats survived. By contrast, deaths occurred on days 32, 33 and 38, with seven of 10 (70%) rats reaching the end point in the tada-10 group.

The RV catheterization on day 42 post MCT-injection revealed an aggravated RVSP and a decreased HR in the MCT rats (Fig. 7b). Although the RVSP values were lower in both treatment groups by comparison, the significant decrease was only seen in the masi-15 rats ($P = 0.04$) but not in the tada-10 rats ($P = 0.08$). In addition, the rats in both treatment groups also showed a higher HR than the MCT rats (Fig. 7c). However, neither the masi-15 nor tada-10 rats significantly reduced the RVH compared with that of the MCT rats (Fig. 7d).

Further, the masi-15 and tada-10 rats achieved significantly higher body weight gain after 28 days post-MCT injection than the MCT rats (Fig. 7e). However, the increases became insignificant on day 35, which might be attributable to abdominal and pleural effusions indicating right heart failure that falsely increased the body weight in the MCT-injected rats. Nevertheless, the masi-15 and tada-10 rats had comparable weight gains.

4. Discussion

This study provides new insight into the effects of masitinib, a tyrosine kinase inhibitor versus tadalafil on the treatment of PAH. We demonstrated that masitinib exhibited more potent, dose-dependent preventive properties against pulmonary vascular remodeling and RV hypertrophy than tadalafil. Besides, masitinib also reduced RVSP in rats with monocrotaline-induced PAH, and both the 15 and 50 mg/kg rat dosages are statistically comparable in terms of the anti-remodeling and therapeutic efficacies. By contrast, tadalafil elicited insignificant anti-remodeling properties and did not block the MAPK pathway as what was reported in sildenafil. Further, we also showed that masitinib increased the cGMP level, and more importantly, the long-term treatment with a low-dose masitinib was non-inferior than tadalafil.

All doses of masitinib significantly suppressed the C-KIT and tended to reduce the PDGFR-β mRNA expression in the lungs of the MCT-injected rats. At a high dose (50 mg/kg), the PDGF-B protein expression was also significantly inhibited. Besides, it also potently blocked the expression of CXCR4 mRNA and phosphorylated ERK protein, collectively implying the inhibitory role of masitinib on the MAPK and CXCR4/CXCL12 pathways which drive the PAH pathogenesis [10,37]. By contrast, the tadalafil treatments did not significantly reduce the mRNA levels of the tyrosine kinase receptors nor the CXCR4 and CXCL12. In line with these observations, tadalafil did not block the Raf-1 mRNA and phosphorylated ERK 1/2 protein expression in the rat lungs, indicating that tadalafil had negligible inhibitory effects on the MAPK pathway, as opposed to what was observed in sildenafil [18].

Further, the prevention of pulmonary arterial (PA) muscularization was also more significant in masitinib- than tadalafil-treated rats. The number of remodeled PAs, indicated by the FMPA percentage, was reduced by 10.5, 16.7, and 19.6% following

treatments of 5, 15, and 50 mg/kg of masitinib, respectively. By contrast, the tadalafil therapy of 5 and 10 mg/kg decreased the FMPA percentage by 8.8 and 7.3%, respectively. Besides, the stronger anti-remodeling properties of masitinib than tadalafil were also reflected by the presence of a higher number of thin-walled PAs represented by the NMPA percentage in the masitinib-treated rats. With respect to the cardiac remodeling, tadalafil at 10 mg/kg significantly reduced the right ventricular hypertrophy (RVH), consistent with that of Sawamura et al. [32], but the statistical significance was lost when the RVH was normalized to the body weight ($P = 0.171$). Nonetheless, the tadalafil-treated rats showed overall higher RVH indices and BNP mRNA expression than those treated with masitinib, indicating that tadalafil possesses weaker direct cardiac anti-remodeling abilities.

Despite the less potent cardiopulmonary preventive abilities, tadalafil significantly reduced the RVSP in the MCT-injected rats, in agreement with the results by Sawamura et al. [32] and Egawa et al. [8]. The concurrent increases in lumen area of the 20–50 and 51–100 μm FMPAs and the low pulmonary PDE-5 mRNA expression in the tadalafil-treated rats strengthened our belief that these hemodynamic improvements were mainly attributable to the PDE-5 inhibition-induced PA vasodilation. It is of interest that the masitinib therapy at a 15 mg/kg dose also tended to reduce the PDE-5 mRNA level in the rat lungs, and concomitantly produced significantly higher serum cGMP concentration upon the treatment extension. With respect to this, Alp Ozgur-Akdemira et al. (2011) showed that the binding of imatinib to the receptors tyrosine kinase activated the nitric oxide-cGMP pathway and induced dilation of smooth muscle of human prostate tissue *in vitro* [31]. Further, previous studies have also reported the vasodilator roles of imatinib, nilotinib, and sorafenib [1], suggesting that masitinib

might also cause pulmonary vasodilation by regulating the NO/cGMP pathway. Despite so, imatinib dilated pulmonary venous beds in guinea pigs through cAMP but not cGMP release [26]. With respect to this, the absence of PDE-5 mRNA downregulation and serum cGMP increase in the rats medicated with a 50 mg/kg dose of imatinib (unpublished data) led us to believe that this discrepancy might be due to pharmacological differences between masitinib and imatinib.

In the present study, the rat dose of 10 mg/kg of tadalafil is equivalent to the clinical dose for PAH in humans, since both produced comparable drug exposure levels (126,000 ng · h/mL in rats versus 121,930 ng · h/mL in humans) [31]. As for masitinib, although the optimal therapeutic dose remains unknown, several cancer clinical studies in humans have evaluated masitinib at a dose between 6–12 mg/kg/day [2,21,25], which gives a dose range of 37.2–74.4 mg/kg in the rats [30]. At 6 weeks post MCT injection, the MCT rats demonstrated signs of heart failure indicated by pleural and abdominal effusion and lower heart rate which could be due to β_1-adrenergic downregulation [35]. In scrutinizing the effects of masitinib versus the human clinical dose of tadalafil for the long-term treatment of PAH, we showed that both treatments significantly increased heart rates of the rats, in which masitinib at a dose as low as 15 mg/kg also tended to delay the mortality onset. More importantly, a much greater and significant reduction in the RVSP was observed in the masitinib- but not the tadalafil-treated rats. Taken together, these data provide strong evidence that masitinib at a low dose was non-inferior and more effective than tadalafil on the long-term control of severe PAH in the rats.

Although masitinib shares similar kinase inhibition profiles with imatinib, it has higher specificity for C-KIT and affinity for PDGFR [6,34]. Unlike imatinib, it lacks

activity for the Bcr-Abl kinase in which the inhibition is believed to cause cardiotoxicity [17], although Hu ~~W~~ et al [16] rebutted that imatinib caused cardiomyocyte dysfunction through induction of endoplasmic reticulum stress and impairment of autophagy. Nevertheless, clinical trials for the use of masitinib in a myriad of non-neoplastic disorders such as amyotrophic lateral sclerosis [29], progressive multiple sclerosis [36], and indolent systemic mastocytosis [25] have revealed favorable outcomes with acceptable safety in the masitinib-treated patients. These proved a high safety margin of masitinib compared with imatinib, more predictably when it is administered at a low dose. While the present study represents a toxin-induced PAH model and lacks complete hemodynamic and in-vitro data to fully support the use of masitinib for the human PAH, the beneficial effects of masitinib as a single agent for the PAH treatment in the rats are clear, and hence the results may serve useful foundation for further clinical investigation on the use of a low-dose masitinib in combination therapy for PAH in humans.

5. Conclusion

Masitinib elicited stronger cardiopulmonary preventive properties than tadalafil, via dual inhibition of the MAPK pathway and PDE-5. The long-term therapy with a lower dose (15 mg/kg) attenuated the PAH severity and improved survival in the rats. Although tadalafil also improved the RVSP in the rats, its weak anti-remodeling abilities did not confer greater long-term survival nor hemodynamic benefits than masitinib. Therefore, a lower dose of masitinib may be applicable in the PAH therapy to target both the cardiopulmonary remodeling and the increased vasoconstriction.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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Ethical Approval

This study was approved by the Institutional Animal Care and Use Committee of the Tottori University (No. 17-T-35).

References

[1] Abe K, Toba M, Alzoubi A, et al. Tyrosine kinase inhibitors are potent acute pulmonary vasodilators in rats. *Am J Respir Cell Mol Biol* 2011; 45: 804–808.

[2] Adenis A, Blay JY, Bui-Nguyen B, et al. Masitinib in advanced gastrointestinal stromal tumor (GIST) after failure of imatinib: a randomized controlled open-label trial. *Ann Oncol* 2014; 25: 1762–1769.

[3] Aggarwal P, Patial RK, Negi PC, et al. Oral tadalafil in pulmonary artery hypertension: a prospective study. *Indian Heart J* 2007; 59: 329–335.

[4] Arita S, Arita N and Hikasa Y. Therapeutic effect of low-dose imatinib on pulmonary arterial hypertension in dogs. *Can Vet J* 2013; 54: 255–261.

[5] Church AC, Martin DH, Wadsworth R, et al. The reversal of pulmonary vascular remodeling through inhibition of p38 MAPK-alpha: a potential novel anti-inflammatory strategy in pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 2015; 309: L333–L347.

[6] Dubreuil P, Letard S, Ciufolini M, et al. Masitinib (AB1010), a potent and selective tyrosine kinase inhibitor targeting KIT. *PLoS One* 2009; 30: e7258.

[7] Duong-Quy S, Bei Y, Liu Z, et al. Role of Rho-kinase and its inhibitors in pulmonary hypertension. *Pharmacol Ther* 2013; 137: 352–364.

[8] Egawa M, Ishikura F, Nishikawa R, et al. Effects of tadalafil to prevent thickening of pulmonary artery in monocrotaline-induced pulmonary hypertension rats: compared with echocardiographic findings: pp.13.485. *J. Hypertens* 2010; 28: e196–e197.

[9] Falk JA, Philip KJ and Schwarz ER. The emergence of oral tadalafil as a once-daily treatment for pulmonary arterial hypertension. *Vasc Health Risk Manag* 2010; 6: 273–280.

[10] Farkas D, Kraskauskas D, Drake JI, et al. CXCR4 inhibition ameliorates severe obliterative pulmonary hypertension and accumulation of C-kit$^+$ cells in rats. *PLoS One* 2014; 9: e89810.

[11] Favre S, Gambini E, Nigro P, et al. Sildenafil attenuates hypoxic pulmonary remodelling by inhibiting bone marrow progenitor cells. *J Cell Mol Med* 2017; 21: 871–880.

[12] Frost AE, Barst RJ, Hoeper MM, et al. Long-term safety and efficacy of imatinib in pulmonary arterial hypertension. *J Heart Lung Transplant* 2015; 34: 1366–1375.

[13] Galiè N, Brundage BH, Ghofrani HA, et al. Tadalafil therapy for pulmonary arterial hypertension. *Circulation* 2009; 119: 2894–2903.

[14] Ghofrani HA, Voswinckel R, Reichenberger F, et al. Differences in hemodynamic and oxygenation responses to three different phosphodiesterase-5 inhibitors in

patients with pulmonary arterial hypertension: a randomized prospective study. *J Am Coll Cardiol* 2004; 44: 1488–1496.

[15] Hatano M, Yao A, Shiga T, et al. Imatinib mesylate has the potential to exert its efficacy by down-regulating the plasma concentration of platelet-derived growth factor in patients with pulmonary arterial hypertension. *Int Heart J* 2010; 51: 272–276.

[16] Hu W, Lu S, McAlpine I, et al. Mechanistic investigation of imatinib-induced cardiac toxicity and the involvement of c-Abl kinase. *Toxicol Sci* 2012; 129:188–199.

[17] Kerkelä R, Grazette L, Yacobi R, et al. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nat Med* 2006; 12: 908–916.

[18] Kiss T, Kovacs K, Komocsi A, et al. Novel mechanisms of sildenafil in pulmonary hypertension involving cytokines/chemokines, MAP kinases and Akt. *PLoS One* 2014; 9: e104890.

[19] Kocic I, Sztormowska K and Jankowski Z. Protective effect of masitinib on cardiovascular function of rats with pulmonary hypertension: gender dependence. *Eur Heart J* 2017; 38: P1342.

[20] Lajoie AC, Bonnet S and Provencher S. Combination therapy in pulmonary arterial hypertension: recent accomplishments and future challenges. *Pulm Circ* 2017; 7: 312–325.

[21] Le Cesne A, Blay JY, Bui BN, et al. Phase II study of oral masitinib mesilate in imatinib-naïve patients with locally advanced or metastatic gastro-intestinal stromal tumour (GIST). *Eur J Cancer* 2010; 46: 1344–1351.

[22] Leong ZP and Hikasa Y. Effects of toceranib compared with sorafenib on monocrotaline-induced pulmonary arterial hypertension and cardiopulmonary remodeling in rats. *Vascul Pharmacol* 2018; 110: 31–41.

[23] Leong ZP, Arita S and Hikasa Y. Long-term effect of low-dose imatinib therapy for pulmonary hypertension due to chronic degenerative mitral valve disease in six dogs. *Thai J Vet Med* 2018; 48: 509–515.

[24] Leong ZP, Okida A, Higuchi M, et al. Reversal effects of low-dose imatinib compared with sunitinib on monocrotaline-induced pulmonary and right ventricular remodeling in rats. *Vascul Pharmacol* 2018; 100: 41–50.

[25] Lortholary O, Chandesris MO, Bulai Livideanu C, et al. Masitinib for treatment of severely symptomatic indolent systemic mastocytosis: a randomised, placebo-controlled, phase 3 study. *Lancet* 2017; 389: 612–620.

[26] Maihöfer NA, Suleiman S, Dreymüller D, et al. Imatinib relaxes the pulmonary venous bed of guinea pigs. *Respir Res* 2017; 18: 32.

[27] Marech I, Patruno R, Zizzo N, et al. Masitinib (AB1010), from canine tumor model to human clinical development: Where we are? *Crit Rev Oncol Hematol* 2014; 91: 98–111.

[28] Montani D, Chaumais MC, Guignabert C, et al. Targeted therapies in pulmonary arterial hypertension. *Pharmacol Ther* 2014; 141: 172–191.

[29] Mora JS and Hermine O. Masitinib as an add-on therapy to riluzole is safe and effective in the treatment of amyotrophic lateral sclerosis (ALS). *J Neurol Sci* 2017; 381: 183.

[30] Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* 2016; 7: 27–31.

[31] Ozgur-Akdemir A, Demirturk K, Karabakan M, et al. Imatinib mesylate (Gleevec) as protein-tyrosine kinase inhibitor elicits smooth muscle relaxation in isolated human prostatic tissue. *Urology*. 2011; 78: 968.e1–6.

[32] Sawamura F, Kato M, Fujita K, et al. Tadalafil, a long-acting inhibitor of PDE5, improves pulmonary hemodynamics and survival rate of monocrotaline-induced pulmonary artery hypertension in rats. *J Pharmacol Sci* 2009; 111: 235–243.

[33] Shimoda LA and Laurie SS. Vascular remodeling in pulmonary hypertension. *J Mol Med (Berl)* 2013; 91: 297–309.

[34] Soria JC, Massard C, Magné N, et al. Phase 1 dose-escalation study of oral tyrosine kinase inhibitor masitinib in advanced and/or metastatic solid cancers. *Eur J Cancer* 2009; 45: 2333–2341.

[35] Sun F, Lu Z, Zhang Y, et al. Stage-dependent changes of β2-adrenergic receptor signaling in right ventricular remodeling in monocrotaline-induced pulmonary arterial hypertension. *Int J Mol Med* 2018; 41: 2493–2504.

[36] Vermersch P, Benrabah R, Schmidt N, et al. Masitinib treatment in patients with progressive multiple sclerosis: A randomized pilot study. *BMC Neurol* 2012; 12: 36.

[37] Young KC, Torres E, Hatzistergos KE, et al. Inhibition of the SDF-1/CXCR4 axis attenuates neonatal hypoxia-induced pulmonary hypertension. *Circ. Res* 2009; 104: 1293–1301.

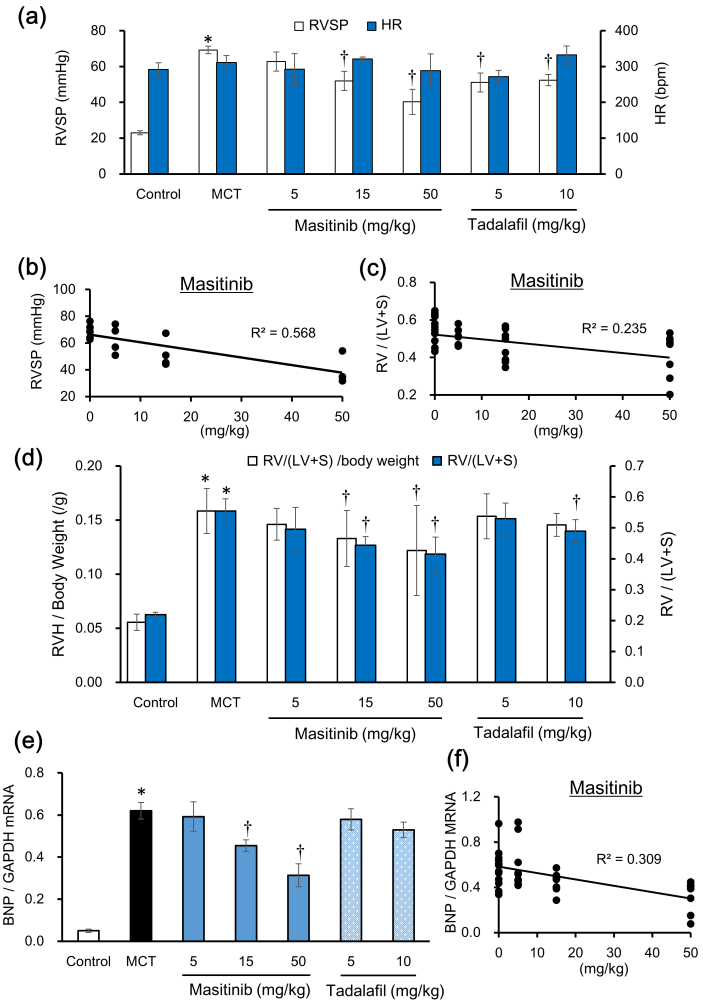

Fig. 1. Effects of masitinib and tadalafil on right ventricular (RV) hemodynamics and remodeling. (a) RV systolic pressure (RVSP) (n=3–6) and heart rate (HR) (n=3–6), (b,c) dose-dependency curves of masitinib on (b) RVSP (n=3–6) and (c) RV hypertrophy (RVH) (n=8–12), (d) RVH indicated by RV /left ventricle and septum (LV+S) weights and RV/(LV+S) normalized to body weight of the rats, (e) mRNA expression of b-type natriuretic peptide (BNP) normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (n=7–14), and (f) dose-dependency curves of masitinib on BNP. Data are means±standard error of mean (SEM). *P<0.05 versus control, †P<0.05 versus MCT.

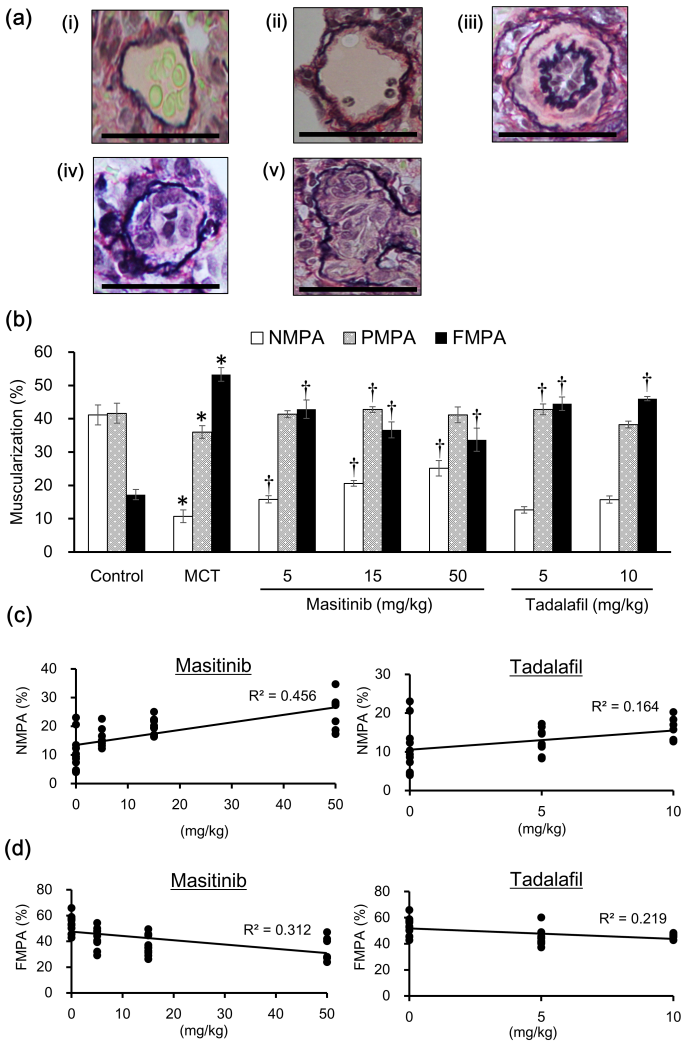

Fig. 2. Effects of masitinib and tadalafil on remodeling of the 20–50 μm pulmonary arteries (PAs). (a) Representative micrographs of (i) non-muscularized (NMPA), (ii) partially (PMPA), (iii) fully muscularized (FMPA), and (iv, v) occlusive PAs which were stained with elastic van Gieson. (b) Muscularization percentage is given by the number of FMPA, PMPA, and NMPA normalized to the total number of PAs counted. Dose-dependency of masitinib on (c) NMPAs and (d) FMPAs. Data are means±SEM (n=8–11). *P<0.05 versus control, †P<0.05 versus MCT.

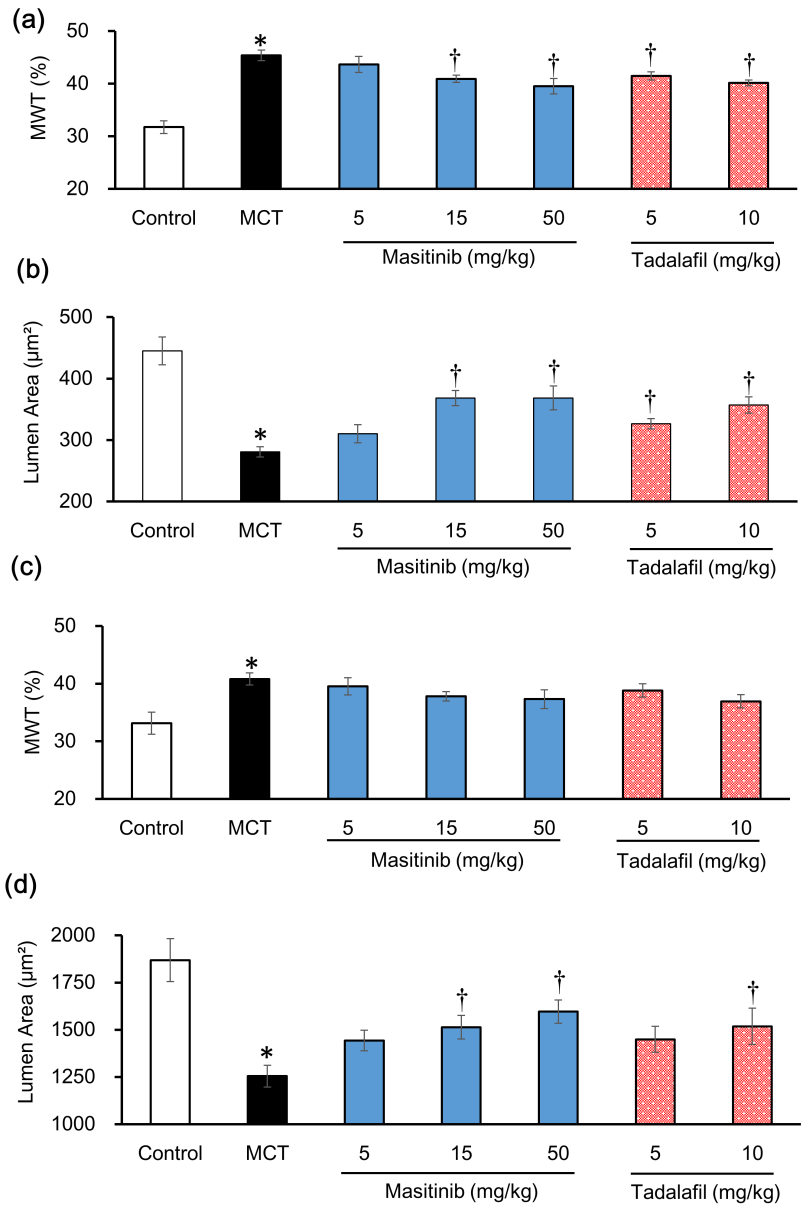

Fig. 3. Effects of masitinib and tadalafil on the (a, c) medial wall thickness (MWT) and (b, d) lumen area of (a, b) 20–50 μm and (c, d) 51–100 μm fully muscularized pulmonary arteries (FMPAs). Data are means±SEM (n=8–12). *P<0.05 versus control, †P<0.05 versus MCT.

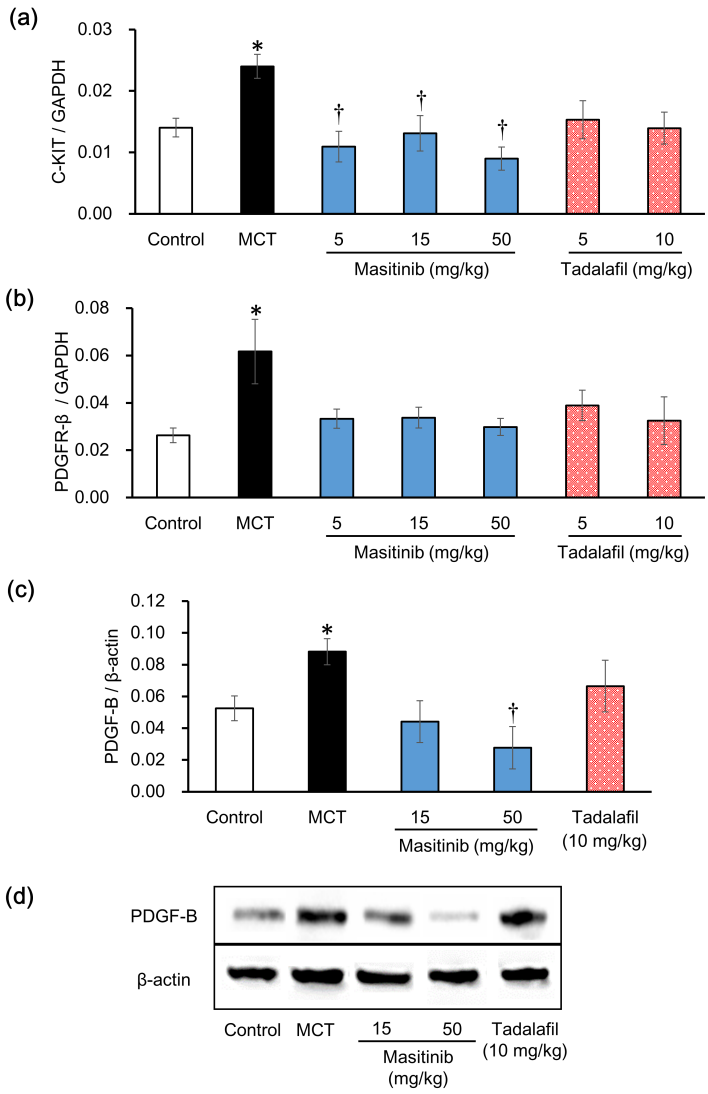

Fig. 4. Effects of masitinib and tadalafil on the expression of (a) KIT proto-oncogene receptor tyrosine kinase (C-KIT) (n=8–12), (b) platelet-derived growth factor (PDGF) receptor-β (PDGFR-β) (n=7–11) mRNAs, and (c) PDGF-B protein (n=3–5) in the rat lungs. (d) Western blots represent PDGF-B versus β-actin proteins of one individual from each treatment group. Data are means±SEM. *P<0.05 versus control, †P<0.05 versus MCT.

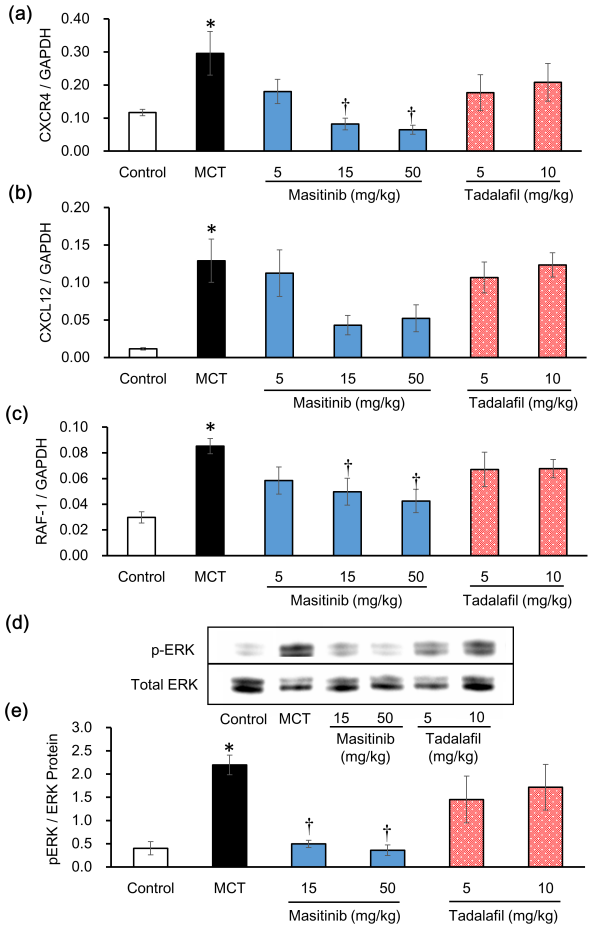

Fig. 5. Effects of masitinib and tadalafil on the C-X-C chemokine receptor type 4 (CXCR4) / CXC ligand (CXCL) 12 and mitogen-associated protein kinase (MAPK) pathways in the rat lungs. (a) CXCR4 mRNA (n=7–9), (b) CXCL12 mRNA (n=8–10), (c) Raf-1 proto-oncogene serine/threonine kinase (Raf-1) mRNA expression (n=8–9), (d) western blots represent phosphorylated versus total ERK protein of one individual from each treatment group (n=4–6), and (e) phosphorylated versus total extracellular-signal-related kinase (ERK)-1/2 protein. Data are means±SEM. *P<0.05 versus control, †P<0.05 versus MCT.

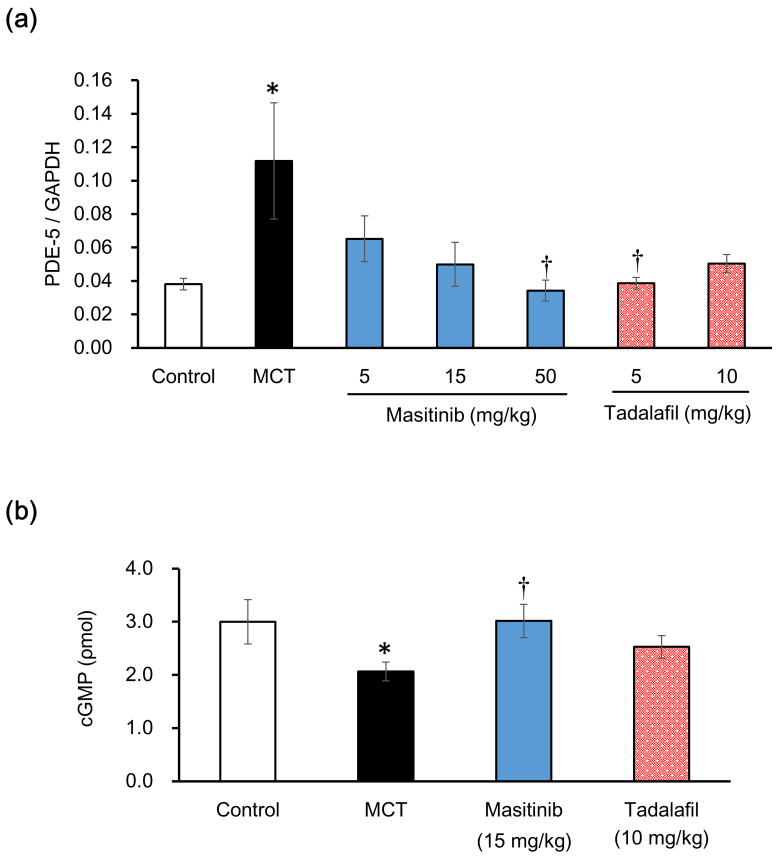

Fig. 6. Effects of masitinib and tadalafil on the nitrogen oxide/cyclic guanosine monophosphate (cGMP) pathway. (a) Expression of phosphodiesterase (PDE)-5 mRNA after 28 days (n=8–9) and (b) serum cGMP levels after 42 days of the monocrotaline (MCT) injection (n=4–11). Data are means±SEM. *P<0.05 versus control, †P<0.05 versus MCT.

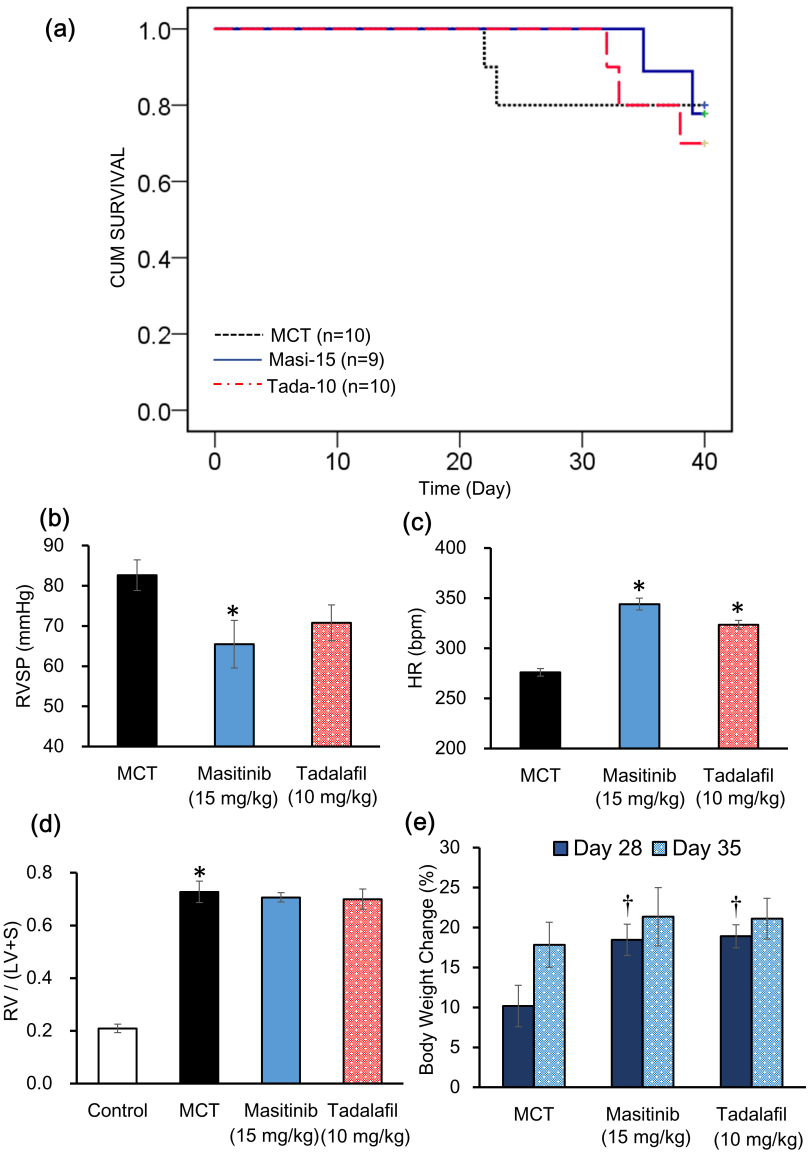

Fig. 7. Comparison of therapy outcomes in the rats treated for 28 days with masitinib (15 mg/kg) versus tadalafil (10 mg/kg). (a) Survival curve with an end point on day 40 post-monocrotaline (MCT) injection (n=9–10), (b) right ventricular (RV) systolic pressure (RVSP) (n=5–6), (c) heart rate (HR) (n=5–6), and (d) RV hypertrophy of the rats (n=6–9), and (e) body weight gain of the rats (n=8–10). Data are means±SEM. *P<0.05 versus control, †P<0.05 versus MCT.