Right ventricular (RV) dysfunction, caused by severe pulmonary hypertension (PH), is associated with high mortality because of RV failure. However, some patients can suffer from sudden cardiac death (SCD). We hypothesized that severe PH can cause RV arrhythmogenesis, leading to SCD. We sought to investigate arrhythmogenesis in PH. Optical mapping analysis (OMP) with an electrophysiologic study (EPS) and pathological examination were performed in a monocrotaline (MCT)–induced rat PH model. Rats were injected with MCT (60 mg/kg), and OMP was performed in isolated Langendorff-perfused hearts. OMP revealed abnormal RV conduction delays and abnormal patterns, along with elevated RV pressure. In addition, impaired action potential duration dispersion (APDd), an index of myocardial repolarization instability, was observed only in the RVs with severe PH. The EPS demonstrated that lethal arrhythmias were induced by burst pacing to the RV when deteriorated APDd became evident. This arrhythmogenesis was inhibited by combination treatment with sildenafil and beraprost (SIL + BERA). RT-PCR showed an mRNA up-regulation of Type I collagen and down-regulation of connexin-43 in the RV at 5 weeks after MCT injection. Pathological examination revealed pulmonary vascular remodeling and RV hypertrophy with interstitial fibrosis, which was substantially reduced by SIL + BERA. Immunohistochemistry also revealed connexin-43 degradation in the RVs with severe PH. In contrast, connexin-43 was well preserved, and no lethal arrhythmias were induced by burst pacing to the RV in the absence of PH after SIL + BERA. In conclusion, RV electrical remodeling, including impaired APDd, causes arrhythmogenesis in severe PH, potentially associated with SCD attributable to PH.

Keywords: pulmonary artery; monocrotaline; optical mapping; arrhythmia

The prognosis of idiopathic pulmonary arterial hypertension has dramatically improved in the past two decades after the emergence of three different drug types for its treatment: prostacyclin, endothelin receptor antagonists, and phosphodiesterase-5 inhibitor (1–6). However, the prognosis of immobile patients with severe pulmonary hypertension (PH) remains quite poor. For example, patients regarded as functional Class IV according to the World Health Organization classification have a mean survival time of approximately 6 months (7, 8). The most common reported cause of death in these cases is right ventricular (RV) failure. Intense RV pressure overload, caused by severe PH, compromises left ventricular (LV) diastolic and systolic function, which consequently induces the systemic condition known as “RV failure” (12, 13). Because the development of RV failure in patients with pulmonary arterial hypertension is often related to untoward outcomes, the prevention and treatment of RV failure are important in severe PH, and in ameliorating obliterated pulmonary vasculature (9–11).

Some patients who suffer from pulmonary arterial hypertension are also reported to experience sudden cardiac death (SCD) because of unknown mechanisms. Kuriyama reported that most patients with severe pulmonary arterial hypertension died of right heart failure (48.6%), SCD (26.4%), and respiratory failure (5.0%) in Japan (14). Lethal arrhythmias such as ventricular tachycardia (VT) or ventricular fibrillation (VF) may be involved in SCD attributable to severe PH, although the precise mechanism remains unknown. To the best of our knowledge, very few reports have investigated RV electrical remodeling and the arrhythmogenic properties of PH.

As an arrhythmogenic substrate, connexin-43, a gap junction protein, plays an important role in myocardial conduction (30, 31). Connexin-43 degradation is known to be responsible for RV electrical remodeling in monocrotaline (MCT)–induced PH (28, 29). In addition, collagen deposition caused by inflammatory response after myocardial infarction or myocarditis serves as an arrhythmogenic substrate to which lethal arrhythmia in both ventricles has been attributed (31, 32).

We hypothesized that PH-induced RV pressure overload causes RV electrical instability and lethal ventricular arrhythmia. Thus, this study investigated whether RV pressure overload influences myocardial arrhythmogenesis in MCT-induced PH. For this purpose, we performed physiological and pathological examinations in the MCT–induced rat PH model. Furthermore, to confirm our hypothesis, we studied the effects of combination treatment with sildenafil and beraprost on...
arrhythmogenesis, because a combination treatment with phosphodiesterase-5 inhibitor and other drugs has been shown effective for PH (17–20). We selected a combination treatment with sildenafil and beraprost in particular because they have been conventionally used in both clinical and experimental PH (17, 41).

MATERIALS AND METHODS

A more detailed methodology is provided in the online supplement.

Animal Models

Sprague-Dawley rats (male, 8 wk old, 250–300 g, n = 69) were subcutaneously injected with MCT (60 mg/kg; Sigma, St. Louis, MO) or an equivalent volume of saline (n = 10), as previously described (21–23). Rats were then killed at 2, 3, 4, 5, or 6 weeks after MCT injection (n = 7 or 10). Normal control rats were killed 5 weeks after saline injection. All animals were treated in compliance with the Guide for the Care and Use of Laboratory Animals (publication no. 85–23, revised 1985, National Institutes of Health, Bethesda, MD).

Cardiac Function on Ultrasound and Pathological Morphology

Ultrasound echocardiograms (UF-750XT; Fukuda Denshi Co. Ltd., Tokyo, Japan) were recorded to evaluate cardiac function. The weight ratios of RV/LV + interventricular septum [IVS]) and RV/body weight were calculated as indices of RV hypertrophy, using excised hearts.

Optical Mapping Analysis

After anesthesia, the heart was rapidly excised for the Langendorff preparation, perfused with oxygenated tyrode solution at 37°C, and stained with the voltage-sensitive dye di-4-Amino-naphthyl-ethylpyridinium (ANEPPS: C28H36N2O3S) (15 µmol/L) for 2 minutes. Optical mapping analysis (OMP) was recorded with a high-quality charge-coupled device camera (Leica, Geneva, Switzerland) for 4 seconds. The action potential over a single beat of both ventricles was quantified by commercial software (Ultima-6006; Sei Media, Inc., Tokyo, Japan). The action potential duration dispersion (maximum action potential duration - minimum action potential duration) was calculated with the formula: action potential duration dispersion. However, survival gradually decreased after the cessation of SIL + BERA treatment. Careful observation of each rat revealed that some rats died unexpectedly, with no apparent signs of either heart failure or acute illness.

Hemodynamics

Hemodynamic data revealed that systolic RV pressure was maintained until 2 weeks, but then increased week by week from 3 weeks to 5 weeks after MCT injection. Moreover, systolic RV pressure significantly decreased at 6 weeks, compared with that at 5 weeks (Table 1).

Cardiac Function According to Ultrasound Echocardiogram and Cardiac Morphology

The weight ratios of RV/(LV + IVS) and RV/body weight remained normal at 2 and 3 weeks after MCT injection. However, they began to increase significantly at 4 weeks after MCT injection, and continued to worsen (Table 1). Both

**Figure 1.** Kaplan-Meier survival curve. The survival rate of rats in normal control and MCT-SIL + BERA groups was statistically better than that in the MCT-No treatment group (P < 0.001, log-rank test). BERA, beraprost; MCT, monocrotaline; MCT-No treatment, MCT without SIL + BERA treatment (n = 23 per group); MCT-SIL + BERA, MCT with SIL + BERA treatment group; Normal control, normal control group; SIL, sildenafil. *P < 0.01 compared with other two groups; *P < 0.01 compared with other two groups.

Quantitative RT-PCR

PCR primers for the target genes and the endogenous control included glyceraldehyde-3-phosphate-dehydrogenase, collagen Type 1-α2, and gap junction protein–α5 (connexin-43).

Immunohistochemistry

Immunohistochemistry was performed using mouse monoclonal antibodies directed against connexin-43, proliferative cell nuclear antigen (PCNA), Ki-67, and α-smooth muscle actin (α-SMA).

Statistical Analysis

Values are presented as means ± SDs. A Kaplan-Meier survival curve to compare three groups was statistically analyzed by log-rank test. Statistical analysis was performed using multiple comparisons with one-way ANOVA, followed by the Bonferroni post hoc test to compare groups. P < 0.05 was considered statistically significant.

RESULTS

Kaplan-Meier Survival Curve

In MCT-induced PH, significant differences were observed among three groups, namely, normal control and MCT with or without SIL + BERA treatment (Figure 1, P < 0.001, n = 23 per group). Daily administration of SIL + BERA for 3 weeks (between Day 15 and Day 35) significantly prolonged survival after MCT injection. However, survival gradually decreased after the cessation of SIL + BERA treatment. Careful observation of each rat revealed that some rats died unexpectedly, with no apparent signs of either heart failure or acute illness.

**Figure 1.** Kaplan-Meier survival curve. The survival rate of rats in normal control and MCT-SIL + BERA groups was statistically better than that in the MCT-No treatment group (P < 0.001, log-rank test). BERA, beraprost; MCT, monocrotaline; MCT-No treatment, MCT without SIL + BERA treatment (n = 23 per group); MCT-SIL + BERA, MCT with SIL + BERA treatment group; Normal control, normal control group; SIL, sildenafil. *P < 0.01 compared with other two groups; *P < 0.01 compared with other two groups.
TABLE 1. RIGHT VENTRICULAR HEMODYNAMICS AND MORPHOLOGICAL CHANGES AFTER MCT INJECTION WITH OR WITHOUT SIL + BERA TREATMENT

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (n = 10)</th>
<th>MCT 2w (n = 7)</th>
<th>MCT 3w (n = 10)</th>
<th>MCT 4w (n = 7)</th>
<th>MCT 5w (n = 10)</th>
<th>MCT 6w (n = 10)</th>
<th>MCT 5w SIL + BERA 3w (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>374 ± 18</td>
<td>351 ± 14</td>
<td>389 ± 14</td>
<td>368 ± 27</td>
<td>337 ± 54</td>
<td>288 ± 11*</td>
<td>348 ± 14</td>
</tr>
<tr>
<td>sRVP, mm Hg</td>
<td>26.9 ± 3.6</td>
<td>25.9 ± 4.8</td>
<td>54.7 ± 5.6*</td>
<td>79.1 ± 7.1*</td>
<td>89.1 ± 6.2*</td>
<td>74.1 ± 4.2*†</td>
<td>26.7 ± 3.7</td>
</tr>
<tr>
<td>mRVP, mm Hg</td>
<td>5.3 ± 1.0</td>
<td>5.2 ± 2.4</td>
<td>8.2 ± 2.1*</td>
<td>10.5 ± 0.8*</td>
<td>12.2 ± 1.1*</td>
<td>11.5 ± 1.6*</td>
<td>5.8 ± 2.0</td>
</tr>
<tr>
<td>IVS paradox, %</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.39 ± 0.03*</td>
<td>0.60 ± 0.12*</td>
<td>0.77 ± 0.12*†</td>
<td>0.30 ± 0.13</td>
</tr>
<tr>
<td>RV/LV</td>
<td>0.23 ± 0.02</td>
<td>0.25 ± 0.03</td>
<td>0.30 ± 0.03</td>
<td>0.91 ± 0.16*</td>
<td>1.25 ± 0.25*</td>
<td>1.97 ± 0.29*†</td>
<td>0.63 ± 0.15</td>
</tr>
<tr>
<td>APD disp, ms</td>
<td>16.5 ± 1.3</td>
<td>17.8 ± 1.7</td>
<td>28.8 ± 3.0*</td>
<td>34.8 ± 3.1*</td>
<td>34.3 ± 2.6*</td>
<td>ND</td>
<td>21.8 ± 2.6</td>
</tr>
<tr>
<td>RV/LV + Sep</td>
<td>0.69 ± 0.12</td>
<td>0.56 ± 0.13</td>
<td>0.63 ± 0.14</td>
<td>0.91 ± 0.16*</td>
<td>1.25 ± 0.25*</td>
<td>1.97 ± 0.29*†</td>
<td>0.63 ± 0.15</td>
</tr>
<tr>
<td>Cond time, ms</td>
<td>15.9 ± 3.6</td>
<td>17.8 ± 1.7</td>
<td>28.8 ± 3.0*</td>
<td>34.3 ± 2.6*</td>
<td>ND</td>
<td>21.8 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>RV/BW</td>
<td>0.24 ± 0.02</td>
<td>0.25 ± 0.03</td>
<td>0.30 ± 0.03</td>
<td>0.91 ± 0.16*</td>
<td>1.25 ± 0.25*</td>
<td>1.97 ± 0.29*†</td>
<td>0.63 ± 0.15</td>
</tr>
<tr>
<td>sRVP, systolic right ventricular pressure</td>
<td>24.8 ± 5.2</td>
<td>25.9 ± 4.8</td>
<td>54.7 ± 5.6*</td>
<td>79.1 ± 7.1*</td>
<td>89.1 ± 6.2*</td>
<td>74.1 ± 4.2*†</td>
<td>26.7 ± 3.7</td>
</tr>
<tr>
<td>APD ind, %</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.39 ± 0.03*</td>
<td>0.60 ± 0.12*</td>
<td>0.77 ± 0.12*†</td>
<td>0.30 ± 0.13</td>
</tr>
<tr>
<td>VT/VF induc, %</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.39 ± 0.03*</td>
<td>0.60 ± 0.12*</td>
<td>0.77 ± 0.12*†</td>
<td>0.30 ± 0.13</td>
</tr>
<tr>
<td>SIL + BERA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.39 ± 0.03*</td>
<td>0.60 ± 0.12*</td>
<td>0.77 ± 0.12*†</td>
<td>0.30 ± 0.13</td>
</tr>
</tbody>
</table>

Definitions of abbreviations: APD disp, action potential duration dispersion; BERA, beraprost; BW, body weight at death; Cond time, right ventricular conduction time; IVS paradox, paradoxical motion of interventricular septum detected by ultrasound echocardiogram; MCT, monocrotaline; mRVP, mean right atrial pressure; ND, not detected; RV/BV, the ratio of right ventricular weight/body weight; RV/LV, the ratio of right ventricular weight/left ventricular plus septal weight; SIL, sildenafil; sRVP, systolic right ventricular pressure; VT/VF induc, induction of ventricular tachycardia and ventricular fibrillation; w, weeks.

Results are presented as means ± 5Ds.

*p < 0.01 indicates statistical significance compared with normal control rats, 2 weeks after MCT injection, or 5 weeks after MCT injection in combination with 3-week administration of SIL + BERA.

1p < 0.05 indicates statistical significance, compared with 5 weeks after MCT injection.

1p < 0.01 indicates statistical significance compared with normal control rats, and with rats 2 weeks or 3 weeks after MCT injection, or 5 weeks after MCT injection treated with 3-week administration of SIL + BERA.

M-mode and B-mode scans of ultrasound echocardiograms indicated no apparent RV dilatation, and moreover, LV function was well-preserved in normal control rats and at 2 and 3 weeks after MCT injection. After PH occurred, however, the RV lumen was marginally but not significantly visible in B-mode scan at 3 weeks, and became more dilated at 4 and 5 weeks after MCT injection (Figures 2A–2E). Furthermore, paradoxical motion of the IVS was apparent at 4 and 5 weeks. Because anesthesia was likely to be fatal to most rats at 6 weeks after MCT injection, a sufficient number of ultrasound–echocardiogram findings could not be obtained at the 6-week point.

In contrast, RV dilatation was substantially improved by a daily administration of SIL + BERA for 3 weeks (Figure 2F), with a marginally visible RV lumen in B-mode scan, which was similar to our findings 3 weeks after MCT injection.

Electrocardiogram Findings

After PH occurred, the electrocardiogram pattern changed week-by-week from normal QRS morphology to a widening QRS morphology at 3 weeks after MCT injection. However, QRS morphology was altered to demonstrate an intraventricular conduction disturbance pattern between 4 and 6 weeks after MCT injection. In addition, low voltage in the QRS, atrioventricular block, and sinus bradycardia with QT elongation were also frequently observed at 5 and 6 weeks after MCT injection (Figures 1 and 2, and Figures 4C, 4D, 4H, and 4I), with an accompanying action potential duration dispersion (Figures 4A–4D).

The serial changes in action potential duration dispersion are summarized in Tables 1 and 2, and a representative case is shown in Figure 4. Action potential duration dispersion was within normal range at 2 and 3 weeks after MCT injection (Figures 4A, 4B, 4F, and 4G), and was also normal with a daily administration of SIL + BERA for 3 weeks (Figures 4E and 4I). In contrast, action potential duration dispersion significantly increased at 4 and 5 weeks after MCT injection (Tables 1 and 2, and Figures 4C, 4D, 4H, and 4I), with an accompanying action potential duration elongation (Figures 4A–4D).

Electrophysiological recording revealed that lethal arrhythmia (VT/VF) was never provoked by burst pacing stimulus to the RV in normal control rats or at 2 weeks after MCT injection (Tables 1 and 2, and Figures 5A–5C). However, VT/VF was easily provoked by moderate (50 V) or maximum (100 V) burst pacing stimulus to the RV at 4 or 5 weeks after MCT injection (Figure 5D). Moreover, VT/VF occurred spontaneously in isolated Langendorff hearts, and VT/VF was more easily induced by the first minimum stimulus (5 V) to the RV at 5 weeks after MCT injection (Figure 5E). Again, because anesthesia was likely to be fatal to most rats at 6 weeks after MCT injection, neither optical mapping data nor an electrophysiological recording was obtained at the 6-week point.

The changes to the time course of action potential duration dispersion were almost identical to those of the induction of lethal arrhythmias by burst pacing stimulus. The behavior of action potential duration dispersion was closely related to that of the induction probability of VT/VF (Tables 1 and 2). Again, combination treatment with SIL + BERA significantly suppressed action potential duration dispersion prolongation, and completely inhibited the inducibility of lethal arrhythmia (Figures 4E, 4J, and 5F).

Quantitative RT-PCR

Quantitative RT-PCR demonstrated that Type 1 collagen mRNA was up-regulated and connexin-43 mRNA was conversely down-regulated in the RV at 5 weeks after MCT injection (Figures 6A and 6B). These changes in gene expression were significantly inhibited by a 3-week administration of SIL + BERA (Figures 6A and 6B).
Pathology and Immunohistochemistry

Pathological examination demonstrated active pulmonary arterial remodeling and RV hypertrophy with interstitial fibrosis that became evident at 4 weeks and worsened by 5 weeks after MCT injection. In addition, Elastica van Gieson staining revealed a medial hypertrophy of the intra-acinar pulmonary artery, which began to develop in the lung parenchyma at 4 weeks and worsened by 5 weeks after MCT injection (Figure E2). Masson trichrome revealed RV hypertrophy with interstitial fibrosis that became evident at 4 weeks (data not shown), and that also worsened by 5 weeks after MCT injection (Figures 7A–7C and 7E–7G). Reticulin silver impregnation stain, which indicates microscopic Type III collagen fiber assembly, also revealed interstitial fibrosis (Figure 7K). In contrast, SIL + BERA substantially ameliorated pulmonary vascular remodeling (Figure E2), and thereby reduced RV hypertrophy with interstitial fibrosis (Figures 7D, 7H, and 7L). These pathological changes and the effect of SIL + BERA combination treatment coincided with the serial changes in action potential duration dispersion and the inducibility of lethal arrhythmias (Tables 1 and 2).

Figure 2. Representative recordings of ultrasound echocardiograms in normal control rats and at 2, 3, 4, and 5 weeks after monocrotaline injection. Extremely narrow RV lumen and well-preserved LV wall motion were observed in normal control rats (A) and at 2 weeks after MCT injection (B) (MCT 2W). (C) However, marginal RV dilatation was observed at 3 weeks after MCT injection (MCT 3W). Significant RV dilatation was revealed at 4 weeks (MCT 4W) (D) and 5 weeks (E) (MCT 5W) after MCT injection. (D and E) In addition, paradoxical motion of the interventricular septum was observed during these phases. (F) In contrast to D and E, marked RV dilatation and paradoxical motion of the interventricular septum significantly subsided with a 3-week administration of SIL + BERA (MCT 5W SIL + BERA 3W). Yellow and red arrows indicate diastolic and systolic LV diameters respectively. BERA, beraprost; LV, left ventricle; MCT, monocrotaline; RV, right ventricle; SIL, sildenafil.
Immunohistochemistry using anti-α-SMA and anti-PCNA antibodies demonstrated that medial hypertrophy was accompanied by smooth muscle hypertrophy and smooth muscle cell proliferation in intra-acinar pulmonary arteries of the lung parenchyma (Figure E2). Immunohistochemistry also revealed that connexin-43 immunoreactivity was much weaker in hypertensive RVs (Figure 7O, top) compared with normotensive RVs, including those at 5 weeks after MCT treated with a 3-week administration of SIL + BERA (Figures 7M, 7N, and 7P, top). In contrast, immunoreactivity for connexin-43 was well preserved in the LV, even at 5 weeks after MCT injection (Figures 7M–7P, bottom). Again, these pathological changes coincided with the serial changes in action potential duration dispersion and the inducibility of lethal arrhythmias (Tables 1 and 2). In addition, intense immunoreactivity for Ki-67 was observed in both the lung and RV only during the late phase (at 4, 5, or 6 weeks) after MCT injection. Ki-67 immunoreactivity was colocalized with adventitial cells in the intra-acinar pulmonary artery and with spindle or round cells migrating between myocardial surfaces in the RV at 5 weeks after MCT injection. No immunoreactivity for Ki-67 was detected in the LV (Figure E3).

All specimens of the heart at 1, 2, 3, 4, 5, and 6 weeks after MCT injection were pathologically evaluated to confirm whether myocarditis had occurred. Inflammation was not observed in the RV during the early phase (at 1 or 2 weeks), and was low during the middle and late phases (at 3, 4, 5, or 6 weeks) after MCT injection. Immunohistochemistry for Ki-67 was also compatible with the pathological findings. Intense Ki-67 immunoreactivity was colocalized with the nuclei of fibroblasts migrating between the myocardial surfaces in the RV at 4 or 5 weeks after MCT injection (Figure E3).

**DISCUSSION**

Our results have demonstrated that PH-induced RV pressure overload can cause RV electrical instability and lethal arrhythmias in the MCT-induced rat PH model, and combination treatment with SIL + BERA for 3 weeks ameliorates RV arrhythmogenesis, along with preventing severe PH. We defined severe PH as the status that induced RV structural remodeling corresponding to systolic RV pressure >70 mm Hg (MCT at 4, 5, or 6 wk; Table 1). Severe PH induces a marked RV pressure overload. In the present study, significant RV hypertrophy and interstitial fibrosis, in conjunction with collagen deposition and connexin-43 gap-junction remodeling, increased the action potential duration dispersion. Furthermore, vulnerability to lethal arrhythmias began to occur at the same time that severe PH developed (MCT at 4 and 5 wk; Table 1 and Figure 7).

Careful observation of the Kaplan-Meier survival curve after MCT intoxication suggests that some rats possibly suffer from SCD because of lethal arrhythmia caused by severe PH, but not by RV failure. RV failure was generally defined as an irreversible RV condition accompanied by possible myocardial ischemia in the RV or reduced LV filling attributable to RV dysfunction, with marked RV enlargement that impaired both cardiac output and oxygen delivery (9–11). In the present study, we defined RV failure as a condition associated with a dramatic increase in mortality and a decrease in systolic RV pressure attributable to pump failure. Almost all rats died by 6 weeks after MCT injection because of RV failure, unless they were treated with SIL + BERA. Throughout our observation period, however, some rats unexpectedly died without any apparent sign of heart failure, approximately 4 weeks after MCT intoxication, and before RV failure developed (Figure 1).

We defined macroscopic RV structural remodeling as (1) an increase of the weight ratios of RV/(LV + IVS) and RV/body weight, and (2) marked RV dilatation with paradoxical motion of the IVS according to ultrasound echocardiograms (a typical sign of large RV). In addition, we defined microscopic RV
structural remodeling as (3) an up-regulation of Type I collagen mRNA and a down-regulation of connexin-43 mRNA gene expression, and (4) marked fibrosis and cellular proliferation accompanied by Ki-67 protein synthesis within the interstitial layer. Both macroscopic and microscopic structural remodeling coincided with the development of severe PH after MCT intoxication (MCT at 4, 5, and 6 wk; Table 1). We revealed Type I collagen mRNA up-regulation by RT-PCR, Type III collagen deposition by reticulin silver staining, and both types of collagen assembly by Masson-trichrome staining in hypertensive RVs (Figures 6 and 7). Microscopically, interstitial fibrosis in hypertensive RVs was composed of both Type I and Type III collagen (Figures 7C, 7G, and 7K).

The RV conduction velocity and pattern obtained by OMP may be more sensitive for the detection of mild or moderate RV pressure elevations than are morphological changes such as RV dilatation with paradoxical motions of IVS and RV hypertrophy. Both abnormal RV conduction patterns and a broad QRS on electrocardiogram were observed under moderate PH (MCT at 3 wk; Table 1, Figures 3 and E1). These results are consistent with a previous clinical study (7). However, RV conduction abnormality could not be the sole contributor to RV arrhythmogenesis.

**TABLE 2. RIGHT VENTRICULAR CONDUCTION AND ARRHYTHMOGENESIS IN MCT-INDUCED PULMONARY HYPERTENSION**

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>MCT 2w</th>
<th>MCT 3w</th>
<th>MCT 4w</th>
<th>MCT 5w</th>
<th>MCT 6w</th>
<th>MCT 5w SIL + BERA 3w</th>
</tr>
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<tbody>
<tr>
<td>PH</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>RV conduction delay</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>RV dilatation</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>RV hypertrophy</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>APD dispersion</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>VT/VF induction</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Definition of abbreviations: APD, action potential duration; BERA, beraprost; MCT, monocrotaline; PH, pulmonary hypertension; RV, right ventricle; SIL, sildenafil; VF, ventricular fibrillation; VT, ventricular tachycardia; w, weeks.

* indicates normal findings, and + indicates abnormal findings.

**Figure 4.** Representative recordings of action potential curves (A–E) and action potential duration histograms (F–J) from which the action potential duration dispersion was calculated in the right ventricle after monocrotaline injection, with or without combination treatment with sildenafil and beraprost. The action potential duration was elongated at 3, 4, and 5 weeks after MCT injection (B–D) compared with normal control rats (A). Action potential duration histograms revealed that the action potential duration dispersion, defined as maximum action potential duration – minimum action potential duration, was significantly larger at 4 and 5 weeks after MCT injection (H and I) than in normal control rats and at 3 weeks after MCT injection (F and G). Combination treatment with sildenafil and beraprost for 3 weeks (MCT 5W SIL + BERA 3W) substantially prevented the action potential elongation (E) and action potential duration dispersion increase (J). Abbreviations are the same as in Figure 3.
Severe PH or severe RV pressure overload may be key factors in producing arrhythmogenic substrates in the MCT-induced rat PH model. In the present study, we demonstrated that severe RV pressure overload induced interstitial fibrosis and altered gap-junction protein structure in the RV, and ultimately provoked abnormal action potential duration dispersion, leading to lethal arrhythmia induction. Mild or moderate PH that occurred at 3 weeks after MCT injection could induce delayed conduction velocity and abnormal conduction patterns in OMP, as well as QRS morphological changes in electrocardiograms. However, only 10% of rats suffered from lethal arrhythmia at 3 weeks after MCT injection. The therapeutic effects of combination treatment with SIL + BERA also indicate that ameliorating PH can prevent the development of arrhythmogenic substrates in RV. The salutary effect of combination treatment with SIL + BERA not only suppressed lethal arrhythmia induction, but also prevented RV structural remodeling and the impairment of electrophysiological properties assessed by OMP.

![Figure 5. Representative electrophysiological recordings before and after electrical burst stimuli to the right ventricle during Langendorff perfusion. Tracings show normal control rats (A and B), and results at 3 weeks (C), 4 weeks (D), and 5 weeks (E) after MCT injection, and at 5 weeks with a 3-week administration of SIL + BERA (F). Underlining indicates one time burst stimulus period (5, 50, or 100 V, 40-ms intervals, 20 trains, which is equivalent to 800 ms). No sustained ventricular tachycardia (VT) and/or ventricular fibrillation (VF) were provoked by moderate (50 V) or maximum (100 V) burst stimulus in normal control rats (A and B, respectively). (C) An infrequent induction rate of VT and/or VF by stimuli was observed at 3 weeks after MCT injection. (D) However, VT and/or VF were easily provoked by moderate (50 V) or maximum (100 V) burst stimuli at 4 weeks after MCT injection. (E) In addition, VT and/or VF were often observed spontaneously or easily provoked after the first minimum burst stimulus (5 V) at 5 weeks after MCT injection. (F) In contrast, no VT and/or VF were provoked at 5 weeks with a 3-week administration of SIL + BERA. Abbreviations are the same as in Figure 3.](image-url)
structural remodeling, pathological changes characterized by RV hypertrophy with collagen deposition and connexin-43 depletion are crucial for arrhythmogenesis (28–31).

Similar to previous reports (15, 24), our results suggest a close relationship between impaired action potential duration dispersion, an index of myocardial repolarization instability (25–27), and the induction of lethal arrhythmias. Electrophysiological recording revealed that lethal arrhythmia was easily provoked only by burst stimulus to the RV in the presence of increased action potential duration dispersion. Benoist and colleagues also reported MCT-induced PH as an arrhythmogenic substrate in hypertensive RV by recording monophasic action potential durations, using ventricular electrodes (24). When a significant increase of action potential duration dispersion occurred, 100% inducibility of VT/VF by burst stimulus to the RV was observed. Both increased interstitial fibrosis and impaired connexin-43 gap-junction remodeling in RV led to deteriorated action potential duration dispersion, and possibly induced arrhythmogenic substrates for lethal arrhythmias. All these abnormalities were prevented by combination treatment with SIL + BERA for 3 weeks (MCT at 5 wk and SIL + BERA for 3 wk; Table 1, and Figures 7D, 7H, 7L, and 7P). These results support the hypothesis that RV pressure overloads attributable to severe PH are closely related to increased arrhythmogenic substrates in the RV.

RV pressure overload per se may induce RV myocardial fibrosis or connexin-43 gap-junction remodeling, which is related to the arrhythmogenic substrate in the RV. Three factors (i.e., substrate, modulating factors, and trigger) are closely associated with lethal arrhythmia provocation. Substrates, including ventricular fibrosis after acute myocardial infarction and acute myocarditis, are sometimes related to the focus of lethal arrhythmogenesis (29–31). Moreover, modulating factors such as myocardial ischemia, autonomous neural abnormality, and electrolyte imbalance are also necessary to elicit VT/VF (35). Premature ventricular contraction, commonly observed even in healthy individuals, works as a trigger to evoke multiple reentries that cause VT/VF.

We demonstrated that 3 weeks of SIL + BERA treatment inhibited the induction of lethal arrhythmias at 5 weeks after MCT injection. However, 3 weeks of SIL + BERA administration starting from 2 weeks after MCT injection did not reverse disease, but instead constituted a preventive approach. In addition, sildenafil itself exerts a direct beneficial effect on the hypertrophied RV myocardium, because it increases cyclic adenosine monophosphate through phosphodiesterase-3 inhibition, as well as cyclic guanosine monophosphate through phosphodiesterase-5 inhibition (43). In the present study, we initiated SIL, BERA, or both at 2 weeks after MCT injection because extracellular matrix formation and transforming growth factor–β mRNA gene expression occur 2 weeks after MCT injection in the pulmonary arteries (21, 22). However, neither a 3-week administration with sildenafil alone nor beraprost alone exerted the beneficial effect of attenuating elevated RV pressure (data not shown). Therefore, we added an experimental group that received 2 weeks of SIL + BERA administration, starting 3 weeks after MCT injection. In this group, SIL + BERA administration began after RV remodeling was established (data not shown). Contrary to our prediction, no successful result was obtained except for 3-week administration with SIL + BERA starting 2 weeks after MCT injection to inhibit MCT-induced pulmonary vascular remodeling. We concluded that 2-week SIL + BERA administration was not sufficiently potent to reverse the established pulmonary vascular remodeling in MCT-induced PH.

In the MCT-induced PH model, we speculate that (1) myocarditis may partly be involved in structural remodeling of the RV in MCT-induced PH, (2) interstitial fibrosis in conjunction with connexin-43 degradation may be more accelerated by RV pressure overload per se during the late phase of MCT-induced RV remodeling, and (3) the RV structural remodeling underlying severe PH is evidently responsible for arrhythmogenesis in the MCT-induced PH model. We further hypothesize that (4) the most important factor for inhibiting lethal arrhythmogenesis was the improvement in RV overload through a decrease of pulmonary vascular resistance through a 3-week administration of SIL + BERA. According to earlier reports, myocarditis occurs predominantly in the RV after MCT injection (16, 32, 42). We pathologically confirmed inflammatory cell infiltration in the RV, especially at 3, 4, and 5 weeks after MCT injection. Moreover, immunohistochemistry revealed intense immunoreactivity for Ki-67 colocalized with spindle or round cells migrating between the myocardium and interstitium in the RV at 5 weeks after MCT injection (Figure E3). These results suggest that the active cell-cycle turnover predominantly occurred in migrating fibroblasts or mononuclear cells, and not in the myocardium.
We conclude there appeared to be little effect of aging in our experimental results. The age of the rat is evidently related to ventricular conduction velocity and pattern, and action potential duration (27). We performed OMP using younger rats (6, 8, and 10 wk old) and older rats (12, 13, 14, 16, 20, and 24 wk old). OMP demonstrated no significant differences in RV conduction velocity and pattern across ages (data not shown). No action potential duration dispersion was observed when RV pressure was normal. Furthermore, an electrophysiological study revealed no lethal arrhythmogenesis at different ages in the noradrenergic RV (data not shown).

The present study has several limitations. First, RV pressure elevation causes myocardial stretching, which can activate stretch-activated ion channels to provoke abnormal action potential duration dispersion via mechanoelectrical transduction (33–36). The involvement of transient receptor potential channels (37, 38), including stretch-activated ion channels, should be further investigated using other acute PH models such as pneumonectomy, acute pulmonary embolism, or pulmonary arterial banding. Second, although we demonstrated VT/VF induction by burst stimulus in Langendorff perfused hearts (i.e., an ex vivo model), we could not observe spontaneous VT/VF in vivo. Treadmill exercise overload or catecholamine provocation testing will be necessary to induce spontaneous VT/VF in living PH models (39) in a future study. Third, Bogaard and colleagues (10, 40) and Drake and colleagues (9) reported that RV pressure elevations per se do not induce RV failure, because pulmonary arterial (PA) banding induces RV hypertrophy, but...
not RV failure. We speculate that long-term exposure to elevated RV pressure may be necessary to change compensated RV into decompensated RV failure after PA banding. To ascertain whether arrhythmogenesis is induced by RV pressure overload per se, future investigations will be necessary with OMP, using a PA banding model. Finally, SIL + BERA treatment may prevent both PH and RV structural remodeling, and so the results concerning the effects of SIL + BERA treatment should be carefully interpreted. However, as shown in Figure E2, a 3-week administration with SIL + BERA acts indirectly as a treatment through a dependent mechanism for maintaining pulmonary arterial pressure and decreasing RV overload. In conclusion, RV electrical remodeling, including increased action potential duration dispersion, is potentially associated with arrhythmogenesis in MCT-induced rat experimental PH. These results provide further insights into understanding the mechanisms of PH-induced sudden death.

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