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Beta-3 Adrenoreceptor Agonist Mirabegron Improves Right Ventricular Function in a Rat Monocrotaline-Induced Pulmonary Hypertension Model

Pedro Mendes-Ferreira[1,2,3] | Birger Tielemans[1,4,5] | Allard Wagenaar[1] | Caroline Bouzin[6] | Rui Adão[2,7,8] | Peter Pokreisz[9,10] | Delphine De Mulder[11] | Adelino Leite-Moreira[2] | Carmen Brás-Silva[2] | Jean-Luc Balligand[12] | Chantal Dessy[11] | Marion Delcroix[13] | Rozenn Quarck[13] | Catharina Belge[13]

[1]Laboratory of Respiratory Diseases & Thoracic Surgery (BREATHE), Department of Chronic Diseases & Metabolism (CHROMETA), KU Leuven – University of Leuven, Leuven, Belgium | [2]RISE-Health, Department of Surgery and Physiology, Faculty of Medicine of the University of Porto, Porto, Portugal | [3]Paris-Porto Pulmonary Hypertension Collaborative Laboratory (3PH), UMR_S 999, INSERM, Université Paris-Saclay, Le Kremlin-Bicêtre, France | [4]Department of Imaging and Pathology, KU Leuven – University of Leuven, Leuven, Belgium | [5]Institute of Pathology, University Hospital RWTH Aachen, Aachen, Germany | [6]IREC Imaging Platform (2IP, RRID: SCR_023378), Institute of Experimental and Clinical Research (IREC), Université catholique de Louvain (UCLouvain), Brussels, Belgium | [7]Department of Pharmacology and Toxicology, School of Medicine, University Complutense of Madrid, Madrid, Spain | [8]CIBER Enfermedades Respiratorias (Ciberes), Madrid, Spain | [9]Department of Cardiovascular Sciences, KU Leuven – University of Leuven, Campus Gasthuisberg, Leuven, Belgium | [10]Center for Biomedical Research and Translational Surgery, Medical University of Vienna, Vienna, Austria | [11]Pole of Pharmacology and Therapeutics (FATH), Institute of Experimental and Clinical Research (IREC), Université catholique de Louvain (UCLouvain), Brussels, Belgium | [12]Pole of Pharmacology and Therapeutics (FATH), Institute of Experimental and Clinical Research (IREC), and Cliniques Universitaires Saint-Luc, Université catholique de Louvain (UCLouvain), Brussels, Belgium | [13]Laboratory of Respiratory Diseases & Thoracic Surgery (BREATHE), Department of Chronic Diseases & Metabolism (CHROMETA), Clinical Department of Respiratory Diseases, University Hospitals, KU Leuven – University of Leuven, Leuven, Belgium

Correspondence: Catharina Belge (catharina.belge@uzleuven.be)

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ABSTRACT

Considering the beta-3 adrenoceptor agonist, mirabegron, may display vasodilator and cardioprotective properties, we investigated the therapeutic potential of mirabegron in monocrotaline-induced pulmonary hypertension in rats. High-dose mirabegron improved right ventricular function, and endothelium-dependent relaxation in isolated rat pulmonary arteries, suggesting that mirabegron may be beneficial in pulmonary arterial hypertension.

Pulmonary hypertension (PH) is a life-threatening condition characterized by a precapillary pulmonary arteriopathy leading to increased pulmonary vascular resistance (PVR) and pulmonary arterial pressure, progressive right heart failure and ultimately death if left untreated. Currently approved pharmacological interventions attempt to vasodilate the overly constricted pulmonary vasculature and have minimal effect on the right ventricle (RV) reverse remodeling [1]. New drugs such as sotatercept and seralutinib claim to have anti-remodeling effects on the pulmonary arteries and have demonstrated improvement in the RV-pulmonary artery coupling without providing mechanistic insight [2].

Preclinical studies have shown that β-blockers improve RV function in experimental PH, without successful clinical translation to date [3]. Nebivolol, a beta-1 adrenergic receptor blocker

Abbreviations: LV, left ventricle; MCT, monocrotaline; PH, pulmonary hypertension; RV, right ventricle; β3AR, beta-3 adrenergic receptor.

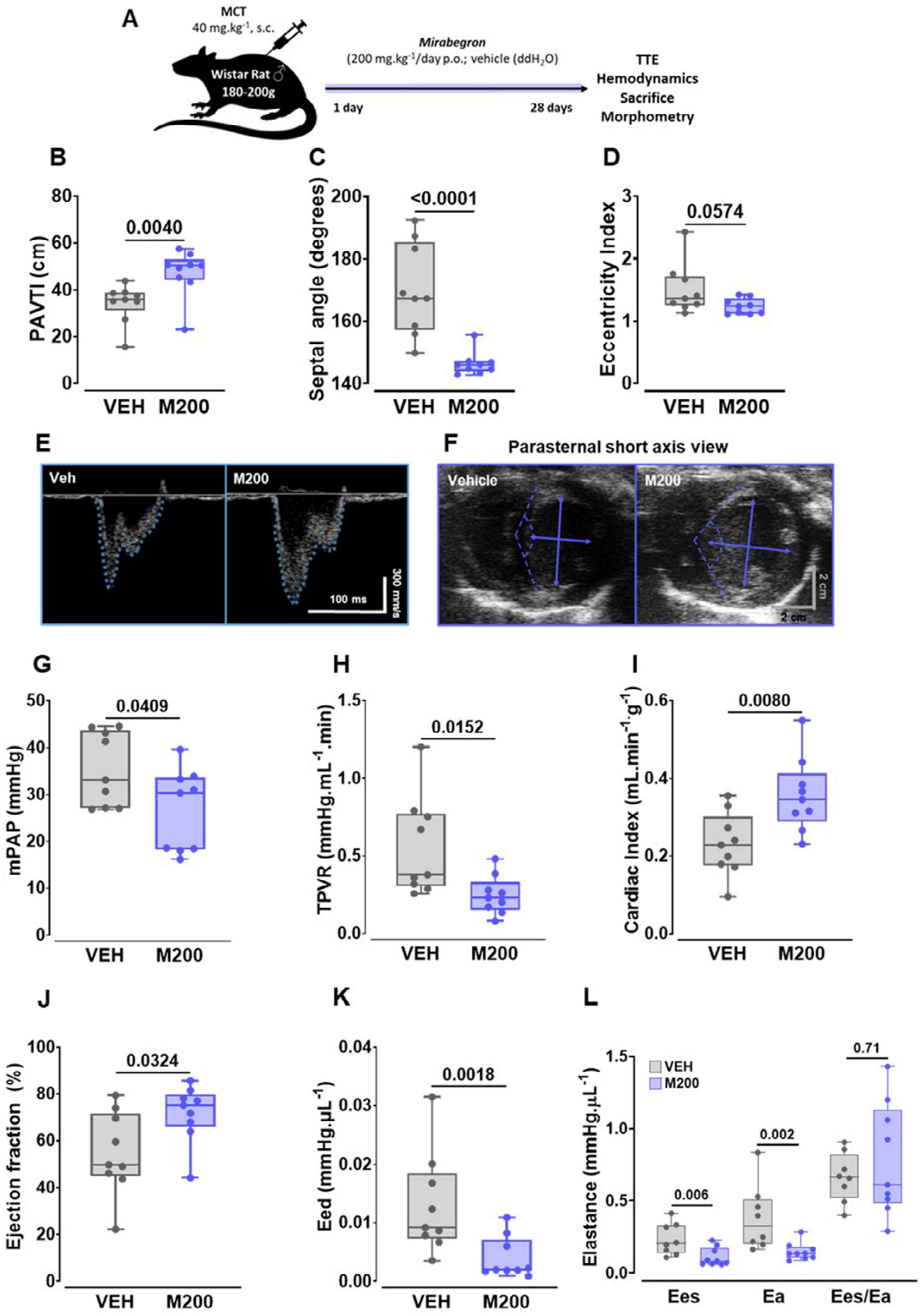

FIGURE 1 | Legend on next page.

and beta-3 adrenergic receptor (β3AR) agonist, restored pulmonary endothelial function, attenuated pulmonary vascular remodeling, and improved RV function in monocrotaline (MCT)-induced PH in rats [4], suggesting a potential benefit of β3AR agonists in PH.

Expression of β3AR has been observed in human, porcine, rat and murine cardiac tissue and pulmonary vasculature [5, 6]. In addition, the β3AR agonist mirabegron, approved for human use in the treatment of overactive bladder, reduced PVR and improved RV performance in a porcine model of chronic postcapillary PH [5]. However, the first clinical trial (SPHERE-HF) with mirabegron failed to reduce PVR in patients with combined post- and precapillary PH [7].

In this research letter, we report the effects of mirabegron in adult male Wistar rats with PH induced by subcutaneous administration of 40 $mg.kg^{-1}$ MCT. Pulmonary hemodynamics and RV function were assessed by transthoracic echocardiography and open-chest cardiac catheterization, performed by placing pressure-volume catheters in the RV and left ventricle (LV) using an apical approach. After sacrifice, second- and third-order pulmonary arteries were studied in a myograph and precontracted with serotonin to avoid unspecific effects associated with the use of α1AR agonists, such as phenylephrine [8]. All animal experiments were performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals and the study protocol was approved by the Institutional Ethics Committee of the KU Leuven (P168/2017).

In a first set of experiments, the presence of β3AR mRNA expression was confirmed in lung tissue from healthy rats and rats with MCT-induced PH ($n = 6$/group) by qRT-PCR, and a similar β3AR mRNA expression was observed in both groups (Figure S1).

In a second set of experiments, the safety and efficacy of different doses (50, 75, or 100 $mg.kg^{-1}$) of mirabegron administered daily per os starting from Day 1 to Day 28 after MCT administration (Figure S2A) were evaluated and compared to vehicle (5% hydroxycellulose) treated animals ($n = 5$–8/group). Of all the doses tested, there was no effect on survival or body weight gain (Figure S2B), and a trend toward decreased mean pulmonary arterial pressure (mPAP) and reduced RV hypertrophy as assessed by the Fulton index (RV/LV + septum weight) (Figure S2C,D).

Therefore, a higher dose of mirabegron (200 $mg.kg^{-1}$; $n = 10$; Figure 1) was compared to vehicle ($n = 9$). Echocardiography showed improvement in pulmonary artery velocity-time integral, unchanged RV and right atrial areas, but decreased septal angle and eccentricity index (Figure 1B–F). At right heart catheterization, mPAP was reduced by 8 mmHg ($p = 0.041$) and total PVR by 55% ($p = 0.015$), while RV ejection fraction and cardiac index were increased by 29% ($p = 0.030$) and 60% ($p = 0.008$), respectively (Figure 1G–J). The pressure–volume relationship showed a decrease in both ventricular ($p < 0.01$) and arterial ($p < 0.01$) elastance, without compromising RV–PA coupling expressed as right ventricular end-systolic (Ees) over pulmonary arterial (Ea) elastance (Ees/Ea) ratio, and an improvement in end-diastolic elastance (Figure 1K,L). The Fulton index was reduced by 24% (0.55 ± 0.09 vs. 0.42 ± 010, $p = 0.008$). In addition, LV ejection fraction was unaffected while LV diastolic stiffness was reduced (Figure S3).

To evaluate the direct effects of mirabegron on the pulmonary vasculature, healthy pulmonary arteries preconstricted with 30 μM serotonin were treated with mirabegron (1–100 μM), which resulted in similar vasodilation in both 2nd and 3rd order pulmonary arteries (Figure S4A,B). The vasodilator effect of mirabegron was independent of pretreatment with a β1/2AR blocker (nadolol; 10 μM) but was reversed by a β1/2/3AR blocker (bupranolol; 10 μM) and was partially dependent on nitric oxide production (inhibitable by L-N^G-Nitro arginine methyl ester) (Figure S4C,D). A similar β3AR agonist-induced vasodilation was observed in both healthy and MCT-induced PH isolated pulmonary arteries (Figure S4E,F). In addition, mirabegron-induced vasodilation was positively correlated with acetylcholine-induced vasorelaxation (tested at the end of the protocol) ($r = 0.7444$, $p = 0.0035$) (Figure S4G), suggesting endothelium-dependent vasodilation by mirabegron in pulmonary arteries. The endothelial nitric oxide synthase (eNOS) dependency of mirabegron fits with its known effects on the vasculature [9] as well as PASMC [5] suggesting it might have an interesting synergistic effect with soluble guanylate cyclase (sGC) stimulators and phosphodiesterase 5 (PDE5) inhibitors. Commonly compromised in pulmonary arterial hypertension (PAH), in both animal models and human samples, eNOS activation can be restored with β3AR agonists [10]. Furthermore, the improved diastolic function, as observed by a significant decreased in end-diastolic elastance (Eed) (Figure 1K) could be explained by increased eNOS signaling [11].

In conclusion, in this preliminary exploratory study, we demonstrate safe and beneficial effects of high-dose mirabegron on pulmonary hemodynamics, RV systolic function, diastolic function and structure, LV diastolic function, and relaxation of isolated pulmonary vessels in a rat model of MCT-induced PH. To our knowledge, this is the first evidence that mirabegron improves both RV systolic and diastolic function in a rat model of MCT-induced PH, and that a β3AR-specific, endothelium-dependent vasodilator response was observed in isolated pulmonary arteries

FIGURE 1 | Effects of high-dose mirabegron on PH development and right ventricular function in monocrotaline (MCT) rats. (A) Schematic of the experimental protocol. Mirabegron (200 mg/kg/day) treatment started 1 day after monocrotaline (MCT) administration and continued until Day 28 post-MCT. (B) Pulmonary artery velocity–time integral (PAVTI), (C) septal angle, (D) eccentricity index, by transthoracic echocardiography (TTE); (E) representative pulmonary arterial flow Doppler signals and (F) representative parasternal short axis view of the heart; arrows represent eccentricity index measurement (anterior–posterior and septal-lateral cavity dimensions) and dashed lines represent the septal angle. (G) Mean pulmonary arterial pressure (mPAP), (H) total pulmonary vascular resistance (TPVR), (I) cardiac index, (J) ejection fraction, (K) end-diastolic elastance, (L) right ventricular end-systolic (Ees), pulmonary arterial (Ea) elastance, RV–PA coupling (Ees/Ea), measured at right heart catheterization before sacrifice in rats treated with vehicle (VEH) and 200 mg/kg/day mirabegron (M200). Data are expressed as median (25th–75th interquartile range). Comparisons by unpaired Student's *t* test (G–I), unpaired Student's *t* test on log-transformed data (D, J, K, L) and nonparametric Mann–Whitney *t* test (B and C).

from healthy and PH animals. This study suggests that the combination of cardioprotective and pulmonary vasodilator effects of the β3AR agonist mirabegron may be beneficial in PAH and supports proof-of-concept studies in humans.

Previous studies have shown that mirabegron has cardioprotective effects on the left heart, limiting myocardial fibrosis and inflammation, and improving LV diastolic function in a rat model of uremic cardiomyopathy [12]. A more recent study also showed RV fibrosis in rats with MCT-induced PH [13], an effect attributed to inhibition of dynamin-related protein 1 and enhanced activation of AMP-activated protein kinase (AMPK). Reduced cardiac fibrosis may explain the prominent effects on RV diastolic function in the current study. Regarding the pulmonary vascular effects, our data demonstrated the specificity of mirabegron for the β3AR receptor, avoiding any potential nonspecific effects on the β1/2AR.

This study has some limitations that merit discussion. While included in initial trials to ascertain model effectiveness and low-dose effects, a healthy control group was not performed simultaneously with the main experimental cohort using high-dose mirabegron 200 mg/kg, which limits interpretation of whether mirabegron restores physiological parameters. Furthermore, we have not performed a control group treated with high-dose mirabegron in order to obtain mirabegron's non-disease related effects. We utilized a preventive approach to better ascertain mirabegron's dose–response in PAH-related hemodynamic effects. Future therapeutic studies will determine its effects in already established disease. The significant improvements in mPAP and cardiac index support a beneficial effect of high dose mirabegron without evidence of toxicity within our treatment window, but does not exclude long-term deleterious effects, which need to be confirmed in future studies.

Due to the lack of a reliable β3AR antibody, we were unable to quantify the expression of this receptor in our model. Although molecular mechanisms were not explored in detail, functional vascular experiments demonstrated nitric oxide–dependent vasodilation, offering relevant mechanistic insight. Finally, the study was conducted only in male rats to simplify this preliminary work.

To move forward, downstream mechanisms such as nitric oxide coupling, oxidative stress, and myocardial fibrosis should be fully explored in further experimental studies. In addition, the optimal dose of mirabegron needs to be investigated, as effects have been reported for a wide range of doses depending on species/strain and route of administration [13]. Future studies should include both sexes to reflect known gender differences in PAH and β3AR expression.

Author Contributions

Pedro Mendes-Ferreira: design of the work, acquisition, analysis and interpretation of data, revised the article critically, approved the version to be published. **Birger Tielemans:** acquisition, analysis and interpretation of data, revised the article critically, approved the version to be published. **Allard Wagenaar:** acquisition, analysis of data, revised the article critically, approved the version to be published. **Caroline Bouzin:** acquisition, analysis and interpretation of data, revised the article critically, approved the version to be published. **Rui Adão:** analysis and interpretation of data, revised the article critically, approved the version to be published. **Peter Pokreisz:** analysis and interpretation of data, revised the article critically, approved the version to be published. **Delphine De Mulder:** acquisition, analysis of data, revised the article critically, approved the version to be published. **Adelino Leite-Moreira:** analysis and interpretation of data, revised the article critically, approved the version to be published. **Carmen Brás-Silva:** analysis and interpretation of data, revised the article critically, approved the version to be published. **Jean-Luc Balligand:** analysis and interpretation of data, revised the article critically, approved the version to be published. **Chantal Dessy:** design of the work; acquisition, analysis and interpretation of data, revised the article critically, approved the version to be published. **Marion Delcroix:** design of the work, analysis and interpretation of data, revised the article critically, approved the version to be published. **Rozenn Quarck:** design of the work, acquisition, analysis and interpretation of data, revised the article critically, approved the version to be published. **Catharina Belge:** concept and design of the work, analysis and interpretation of data, drafted the article and revised it critically, approved the version to be published.

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Ethics Statement

All animal experiments were performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals and the study protocol was approved by the Institutional Ethics Committee of the KU Leuven (P168/2017).

Conflicts of Interest

The authors declare no conflicts of interest.

Pedro Mendes-Ferreira
Birger Tielemans
Allard Wagenaar
Caroline Bouzin
Rui Adão
Peter Pokreisz
Delphine De Mulder
Adelino Leite-Moreira
Carmen Brás-Silva
Jean-Luc Balligand
Chantal Dessy
Marion Delcroix
Rozenn Quarck
Catharina Belge

References

1. M. Humbert, O. Sitbon, C. Guignabert, et al., "Treatment of Pulmonary Arterial Hypertension: Recent Progress and a Look to the Future," *Lancet Respiratory Medicine* 11 (2023): 804–819, https://doi.org/10.1016/S2213-2600(23)00264-3.

2. R. Souza, D. B. Badesch, H. A. Ghofrani, et al., "Effects of Sotatercept on Haemodynamics and Right Heart Function: Analysis of the STELLAR Trial," *European Respiratory Journal* 62 (2023): 2301107, https://doi.org/10.1183/13993003.01107-2023.

3. J. S. J. A. van Campen, K. de Boer, M. C. van de Veerdonk, et al., "Bisoprolol in Idiopathic Pulmonary Arterial Hypertension: An Explorative Study," *European Respiratory Journal* 48 (2016): 787–796, https://doi.org/10.1183/13993003.00090-2016.

4. F. Perros, B. Ranchoux, M. Izikki, et al., "Nebivolol for Improving Endothelial Dysfunction, Pulmonary Vascular Remodeling, and Right Heart Function in Pulmonary Hypertension," *Journal of the American College of Cardiology* 65 (2015): 668–680, https://doi.org/10.1016/j.jacc.2014.11.050.

5. A. García-Álvarez, D. Pereda, I. García-Lunar, et al., "Beta-3 Adrenergic Agonists Reduce Pulmonary Vascular Resistance and Improve Right Ventricular Performance in a Porcine Model of Chronic Pulmonary Hypertension," *Basic Research in Cardiology* 111 (2016): 49, https://doi.org/10.1007/s00395-016-0567-0.

6. S. Moniotte, C. Belge, B. Sekkali, et al., "Sepsis Is Associated With an Upregulation of Functional β3 Adrenoceptors in the Myocardium," *European Journal of Heart Failure* 9 (2007): 1163–1171, https://doi.org/10.1016/j.ejheart.2007.10.006.

7. A. García-Álvarez, I. Blanco, I. García-Lunar, et al., "β3 Adrenergic Agonist Treatment in Chronic Pulmonary Hypertension Associated With Heart Failure (SPHERE-HF): A Double Blind, Placebo-Controlled, Randomized Clinical Trial," *European Journal of Heart Failure* 25 (2023): 373–385, https://doi.org/10.1002/ejhf.2745.

8. H. Kozłowska, E. Schlicker, M. Kozłowski, U. Siedlecka, J. Laudański, and B. Malinowska, "Ligands at β2-, β3-, and the Low-Affinity State of β1-Adrenoceptors Block the α1-Adrenoceptor-Mediated Constriction in Human Pulmonary and Rat Mesenteric Arteries," *Journal of Cardiovascular Pharmacology* 46 (2005): 76–82, https://doi.org/10.1097/01.fjc.0000162775.23139.3e.

9. J. N. Trochu, V. Leblais, Y. Rautureau, et al., "Beta 3-Adrenoceptor Stimulation Induces Vasorelaxation Mediated Essentially by Endothelium-Derived Nitric Oxide in Rat Thoracic Aorta," *British Journal of Pharmacology* 128, no. 1 (1999): 69–76, https://doi.org/10.1038/sj.bjp.0702797.

10. K. K. Galougahi, C. Liu, A. Garcia, et al., "Beta3 Adrenergic Stimulation Restores Nitric Oxide/Redox Balance and Enhances Endothelial Function in Hyperglycemia," *Journal of the American Heart Association* 5 (2016): e002824, https://doi.org/10.1161/JAHA.115.002824.

11. C. Farah, M. Michel LY, and J. L. Balligand, "Nitric Oxide Signalling in Cardiovascular Health and Disease," *Nature Reviews Cardiology* 15, no. 5 (2018): 292–316, https://doi.org/10.1038/nrcardio.2017.

12. Z. Z. A. Kovács, G. Szűcs, M. Freiwan, et al., "Comparison of the Antiremodeling Effects of Losartan and Mirabegron in a Rat Model of Uremic Cardiomyopathy," *Scientific Reports* 11 (2021): 17495, https://doi.org/10.1038/s41598-021-96815-5.

13. L. Zhao, H. Luo, T. Li, X. Zhao, and Y. Liu, "β3 Adrenoceptor Agonist Mirabegron Protects Against Right Ventricular Remodeling and Drives Drp1 Inhibition," *Cardiovascular Diagnosis and Therapy* 12 (2022): 815–827, https://doi.org/10.21037/cdt-22-274.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Figure S1 –β3-adrenoreceptor mRNA expression in lung tissue from healthy and monocrotaline (MCT)-rats. Total RNA was extracted from rat lung tissue and reverse transcripted in DNA. β3-adrenoreceptor mRNA and 18S RNA (housekeeping gene) expression was measured by quantitative real-time polymerase chain reaction using specific primers and Platinum™ SYBR™ Green polymerase. Relative expression was calculated using the comparative ΔCt method: $2^{-(\text{Ct gene of interest-Ct housekeeping gene})}$. Data are expressed as median (25th-75th interquartile range). Comparison by Mann-Whitney t-test. Figure S2 - *In vivo* dose-response of chronic treatment in monocrotaline (MCT)-induced PH. (A) Schematic of the experimental protocol. Mirabegron treatment started 1 day after MCT administration and continued until day 28 post MCT. Mirabegron was administered daily per os (p.o.) at a dose of 50, 75 or 100 $mg.kg^{-1}$. (B) Final body weight. (C) Mean pulmonary arterial pressure (mPAP) at right heart catheterization. (D) Fulton index defined as ratio right ventricle weight/(left ventricle + septum) weight measured at sacrifice 28 days after MCT administration. Data are expressed as median (25^{th}-75^{th} interquartile range). Comparisons by one-way ANOVA, (B, D) or Kruskal-Wallis (C). s.c., sub-cutaneous; TTE, transthoracic echocardiography; VEH, vehicle. Figure S3 – Effects of high-dose mirabegron on left ventricular function. (A) Left ventricular (LV) end-systolic pressure (ESP). (B) LV ejection fraction (EF). (C) LV maximum rate of pressure increase (dP/dtmax). (D) LV end systolic elastance (Ees). (E) LV end-diastolic elastance (Eed) in animals treated with vehicle (VEH) or 200 $mg.kg^{-1}$ mirabegron (M200). Data are expressed as median (25^{th}-75^{th} interquartile range). Comparisons by Student t-test. Figure S4 – Effect of mirabegron on isolated pulmonary arteries. (A) Relaxation curves in response to vehicle and increasing concentration of mirabegron (1-100 μM) and (B) maximum relaxation (Emax) in 2nd and 3rd order pulmonary arteries isolated from healthy animals and pre-constricted with serotonin (5-HT, 30 μM). (C) Relaxation curves in response to vehicle and increasing concentration of mirabegron (1-100 μM) and (D) maximum relaxation (Emax) in 2nd order pulmonary arteries isolated from healthy animals, pre-constricted with serotonin and incubated with vehicle (VEH, ddH2O), mirabegron (MIRA, 100 μM), mirabegron (100 μM) and nadolol (NADO, 10 μM) or bupranolol (BUPRA, 10 μM) or L-NAME (10 μM). (E) Mirabegron dose-response (1-100 μM) and (F) maximum relaxation (Emax) in pulmonary arteries isolated from healthy rats and rats with monocrotaline (MCT)-induced PH. Numbers indicate number of vessels/number of animals (B, F, D), Scatter plot represents individual values and bars represent mean values ± SEM. Comparison by two-way ANOVA repeated measures with post-hoc Tukey's multiple comparisons (A: Group, $p=0.0069$; Dose, $p<0.0001$; Interaction, $p<0.0001$; C: Group, $p<0.0001$; Dose, $p<0.05$; Interaction, $p<0.0001$; E: Group, $p<0,05$; Dose, $p<0.0001$; Interaction, $p<0.0001$); by one-way ANOVA with Holm-Sidak's post-hoc multiple comparisons test (B, D, F). Pearson correlation between mirabegron-induced and acetylcholine-induced vasodilation (G). 5-HT, serotonin. Supplementary Figures.