

Bisoprolol Delays Progression Towards Right Heart Failure in Experimental Pulmonary Hypertension

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Background—In pulmonary arterial hypertension (PH), sympathetic adrenergic activity is highly elevated. Sympathetic overactivity is a compensatory mechanism at first, but might be detrimental for cardiac function in the long run. We therefore investigated whether chronic low-dose treatment with bisoprolol (a cardioselective β -blocker) has beneficial effects on cardiac function in experimental PH.

Methods and Results—PH was induced in rats by a single injection of monocrotaline (60 mg/kg). Pressure telemetry in PH rats revealed that 10 mg/kg bisoprolol was the lowest dose that blunted heart rate response during daily activity. Ten days after monocrotaline injection, echocardiography was performed and PH rats were randomized for bisoprolol treatment (oral gavage) or vehicle ($n=7$ /group). At end of study (body mass loss $>5\%$), echocardiography was repeated, with additional pressure-volume measurements and histomolecular analyses. Compared with control, right ventricular (RV) systolic pressure and arterial elastance (measure of vascular resistance) more than tripled in PH. Bisoprolol delayed time to right heart failure ($P<0.05$). RV afterload was unaffected, however, bisoprolol treatment increased RV contractility and filling (both $P<0.01$), and partially restored right ventriculo-arterial coupling and cardiac output (both $P<0.05$). Bisoprolol restored RV β -adrenergic receptor signaling. Histology revealed significantly less RV fibrosis and myocardial inflammation in bisoprolol treated PH rats.

Conclusions—In experimental PH, treatment with bisoprolol delays progression toward right heart failure, and partially preserves RV systolic and diastolic function. These promising results suggest a therapeutic role for β -blockers in PH that warrants further clinical investigation. (*Circ Heart Fail.* 2012;5:97-105.)

Key Words: pulmonary hypertension ■ right ventricular dysfunction ■ β -adrenergic receptor blocker ■ pressure-volume relationship ■ Wistar rats

Pulmonary arterial hypertension (PH) is a fatal disease, characterized by progressive vascular remodeling and increased right ventricular (RV) afterload, which eventually leads to manifest right heart failure (RHF) and premature death. Current available medical treatments aim to reduce RV afterload, thereby secondarily improving RV function.¹ No treatment is currently available that improves RV function directly, partially because it was not considered a therapeutic target in PH.²

Clinical Perspective on p 105

Recently, several reports have shown that sympathetic activity is increased in patients with PH.³⁻⁷ Similar to left heart failure (LHF), “ventricle-specific” down regulation of β_1 -adrenergic receptors was observed in RV samples of PH patients.⁸ In addition, we recently demonstrated that exercise

training was detrimental in experimental and progressive PH.⁹ The deleterious effects could be related to bouts of exercise-induced sympathetic stimulation.

Although increased adrenergic activity is a compensatory mechanism to maintain cardiac function by increasing contractility and heart rate, it became apparent that chronic adrenergic overactivity has, in the long run, detrimental effects on cardiac function.¹⁰ This supports the use of β -adrenergic blockade in LHF management, which has been demonstrated to significantly reduce mortality and left ventricular (LV) remodeling.¹¹

Nevertheless, and notwithstanding the substantial evidence of their beneficial effects in LHF, the use of β -blockers currently is not recommended for patients with PH.¹ PH patients are unable to increase stroke volume during exercise,

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and as a consequence they are presumed to be highly heart rate dependent to raise cardiac output.¹² Furthermore, in an acute model of PH, it was demonstrated that right ventriculo-arterial uncoupling occurs after intravenous β -blocker administration.¹³

However, the β -blockers used in these studies were first generation unselective β -blockers,^{13,14} with more bronchial and vascular side effects.¹⁰ In addition, the dosages used in these studies were relatively high, whereas a low dose could have sufficed and been tolerated better. Furthermore, no data are available on the long-term effects of β -adrenergic receptor blockade in PH patients. This aspect is important, as the typical time course of improvement by β -blockers in LHF is preceded by initial functional decline, with significant clinical improvement not to be expected before 3 months after start of therapy.¹⁵

Recently, Bogaard and coworkers¹⁶ provided some hemodynamic and molecular insights in effects of carvedilol (an unselective β -blocker) in PH rats. However, in the absence of load independent measurements of RV function, it is unclear whether the beneficial effects are RV-specific or related to the α_1 -associated pulmonary vascular effects of carvedilol.¹⁷ In addition, it remains uncertain whether β -blockers can restore β -adrenergic receptor (β AR) signaling and delay progression of right heart failure. In the present study, we therefore (1) assessed the chronic effect of bisoprolol (a cardio-selective β -blocker) on disease progression by sequential echocardiographic measurements, (2) evaluated RV function using load independent parameters derived from pressure-volume analyses, and (3) studied the β AR- signaling, assessing its direct downstream targets.

Methods

All experiments were approved by the Institutional Animal Care and Use Committee of the VU University, Amsterdam, The Netherlands. Male Wistar rats were used (150 to 175 g, 30 in total), and experimental PH was induced by monocrotaline (60 mg/kg).^{9,18}

Part I: Dose Finding by Pressure Telemetry

The *minimal* effective dose of bisoprolol that could blunt heart rate response during daily activity (>10%) was determined by telemetry (TA11PA-C40, Data Science International [DSI], St. Paul, MN).¹⁹ Ten days after PH induction, bisoprolol was given once daily for 3 consecutive days by oral gavage, at start of their active phase (ie, night: 18:00 to 06:00 hours); 4 PH rats received 5 mg/kg bisoprolol once daily and the other 4 received 10 mg/kg. The effect of bisoprolol on heart rate, systemic blood pressure, and physical activity were evaluated (for details, see the online-only supplement).

Part II: "Clinical" Effects of Bisoprolol Treatment in Experimental PH

In the second part of the study, 22 rats were included (no telemetry): 8 control rats and 14 rats treated with monocrotaline. Ten days after PH induction, PH rats were randomized for bisoprolol treatment (PH+biso; 10 mg/kg) or vehicle/water (PH) by oral gavage (n=7/group). Rats were treated for maximally 3 weeks (day 10 until day 31). Rats that showed clinical signs of manifest RHF (defined as >5% loss in body mass or respiratory distress, cyanosis, lethargy) were euthanized earlier, in keeping with the protocol approved by the Institutional Animal Care and Use Committee. Manifest RHF was a survival end point and recorded as an event in the survival analyses.⁹

Hemodynamic Evaluation

Rats were evaluated by echocardiography 10 days after (monocrotaline) injection and at end of study (when manifest RHF developed, or 31 days after injection).^{9,19} At end of study, open-chest RV catheterization (Millar Instruments, Houston, TX) was performed under general anesthesia in all rats (isoflurane induction: 4.0% in 1:1 O₂/air mix; maintenance: 2.0% in 1:1 O₂/air mix; see the online-only supplement).⁹ Noninvasive estimations of disease progression and RV wall stress are described in the online-only supplement.

Using custom made algorithms (programmed in MATLAB 2007b, The MathWorks, Natick, MA) RV (peak-) systolic pressures and RV end-diastolic pressures were automatically determined from steady state measurements, as well as arterial elastance (Ea), a measure for RV afterload.^{13,20} From occlusion data, end-systolic elastance (Ees; contractility) and end-diastolic elastance (Eed; filling) were determined.^{20,21} These parameters represent the slope of the end-systolic and end-diastolic pressure-volume relationships, respectively, and are considered load independent measures for cardiac contractility (Ees) and filling (Eed).^{20,22} In addition, we calculated the preload recruitable stroke work and the dP/dtmax end-diastolic volume relation to assess RV contractile performance.²³ The ratio Ees/Ea was calculated as an estimate for ventriculo-arterial coupling (cardiac adaptation in relation to its load).^{13,20}

Histomorphology of Heart and Lungs

After the final hemodynamic assessment, all 22 rats were euthanized (by exsanguination under isoflurane), and heart, lungs, and other major organs were harvested. Cardiomyocyte cross-sectional area, cardiac fibrosis, relative wall thickness of pulmonary arterioles, myocardial capillary density (using CD31-antibodies), and myocardial inflammation (using CD45-antibodies) were determined (see the online-only supplement).^{9,24}

Protein Analyses β -Adrenergic Signaling

Phosphorylation of cardiac myosin binding protein C (cMyBPC) and cardiac troponin I (cTnI) was determined as described before.²⁵ All RV samples were treated with trichloroacetic acid, to preserve phosphorylation of cMyBPC and cTnI. Samples were separated on a gradient gel (Criterion Tric-HCL 4% to 15% gel, Bio-Rad Laboratories, Berkeley, CA), and proteins were stained for 1 hour with ProQ Diamond Phosphoprotein Stain (Molecular Probes, Eugene, OR). Fixation, washing, and destaining were performed according to manufacturers' guidelines. Subsequently, gels were stained with SYPRO Ruby staining (Molecular Probes) for determination of total protein levels of cMyBPC and cTnI. The phosphorylation status of cMyBPC and cTnI was expressed relative to total protein levels to correct for differences in sample loading.

Statistical Analysis

All analyses were performed in a blinded fashion. All data were verified for normal distribution. Data are presented as mean \pm SEM. A probability value <0.05 was considered statistically significant.

Comparison of telemetric registrations of PH rats before/after bisoprolol treatment was performed by 2-way ANOVA for repeated measurements, and the interaction between bisoprolol treatment and time was tested and reported. One-way ANOVA was used for the analyses of disease progression, pressure-volume relation, autopsy data, and protein analyses, with Bonferroni posthoc comparison between PH rats with/without bisoprolol treatment. Survival estimates were performed by Kaplan-Meier analysis, with posthoc comparison performed by log rank (Mantel-Cox) test between PH rats with/without bisoprolol treatment (SPSS 16.0 for Windows, SPSS, Chicago, IL).

Histological data were analyzed using multilevel analysis to correct for nonindependence of successive measurements per animal (MLwiN 2.02.03, Center for Multilevel Modeling, Bristol, UK).^{9,24}

Results

Part I: Minimal Effective Dose of Bisoprolol in PH Rats

Echocardiography confirmed the PH status of all 8 rats at day of bisoprolol administration (reduced pulmonary artery ac-

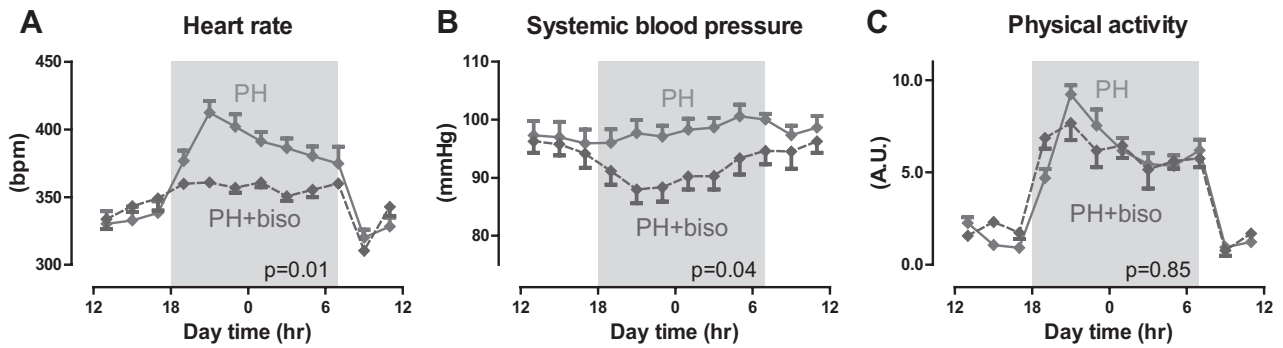


Figure 1. Averaged 24-hour registration of PH rats before and after treatment of 10 mg/kg bisoprolol. This dosage was able to completely blunt heart rate response during the whole active phase (between 18:00 and 06:00 hours) of the rats (**A**: gray area). In addition, only a moderate effect on systemic blood pressure was observed (**B**), without an (adverse) effect on daily physical activity (**C**). Data presented as mean \pm SEM, $n=4$ (PH/PH+biso). Probability values represent interactive term (time*treatment). PH (solid/red line) indicates vehicle-treated PH rats; PH+biso (dotted/blue line), bisoprolol-treated PH rats.

celeration time/cl, increased RV wall thickness). Only 10 mg/kg was able to completely blunt heart rate response during daily activity completely (Figure 1A). At this dose, systemic blood pressure and physical activity were minimally affected, which indicates that this dosage was well tolerated by PH rats (Figures 1B and C).

Part II: Effects of 10 mg/kg Bisoprolol in Established PH

Bisoprolol Delayed the Progression Toward Right Heart Failure

In a separate group of rats, we determined PH status 10 days after (monocrotaline) injection, by echocardiography, right heart catheterization, and histomorphology. Monocrotaline-treated rats ($n=5$) revealed lower PAAT/cl, indicating higher RV systolic pressure⁹ and higher RV wall thickness, indicating (moderate) RV hypertrophy (Table S1; see the online-only supplement). In addition, increased RV systolic pressures, pulmonary vascular remodeling, and RV hypertrophy at day 10 were confirmed by RV catheterization and histomorphometric analyses (Tables S1 and S2; see the online-only supplement). The PH state before start of bisoprolol treatment, thereby, was confirmed in all monocrotaline-treated rats. Compared with vehicle-treated PH rats, bisoprolol delayed the time to manifest RHF, as defined in the Methods section (Figure 2). This finding was confirmed by serial echocardiography, demonstrating that bisoprolol significantly delayed the progression of RV dilatation and reduced the decline in cardiac function, whether measured by tricuspid annular plane systolic excursion or cardiac output (Figure 3; Table S3 [see the online-only supplement]; $P<0.05$). Preservation of cardiac output was mainly the result of an increase in stroke volume rather than increased heart rate.

At end of study, cardiac function was maintained partially by bisoprolol treatment (tricuspid annular plane systolic excursion, control: 4.0 ± 0.1 ; PH: 1.4 ± 0.1 ; PH+biso 2.4 ± 0.2 mm, PH versus PH+biso: $P<0.001$; cardiac output, control: 88 ± 2.1 ; PH 17 ± 1.6 ; PH+biso: 34 ± 1.9 mL/min, PH versus PH+biso: $P<0.001$; Figures 3D and E). No differences were observed in RV wall thickness and RV dilatation between bisoprolol- and vehicle-treated PH rats (Figures 3B and C). In addition, end-systolic wall stress was similar in

bisoprolol- and vehicle-treated PH rats (RV end-systolic wall stress, control: 80 ± 5 ; PH: 336 ± 26 ; PH+biso: 308 ± 14 mm Hg; PH versus PH+biso: $P=0.26$).

Bisoprolol Improved Cardiac Function, Without Affecting RV Afterload

Right ventricular pressure-volume measurements at end of study (Figures 4A–C) revealed that RV systolic pressures were significantly elevated in PH rats compared with control, but no difference was found between bisoprolol- and vehicle-treated PH rats (Figure 4D), which is in line with previous echo findings (Figures 3A and B). Ea (measure of vascular resistance) was elevated also in PH, but again, no difference was observed between bisoprolol- and vehicle-treated PH rats (Figure 4E). This indicates that bisoprolol treatment did not affect RV afterload. We also found an equal rise in (wet) lung mass observed at autopsy, and comparable remodeling of the pulmonary arteries during histological examination (Tables S4 and S5; see the online-only supplement).

On the other hand, bisoprolol treatment increased Ees (measure of contractility; Figure 4F), resulting in partial normalization of the ventriculo-arterial coupling (Ees/Ea; Figure 4G). Preload recruitable stroke work (control: 211 ± 35 , PH: 1362 ± 156 , PH+biso: 2161 ± 312 mm Hg; PH

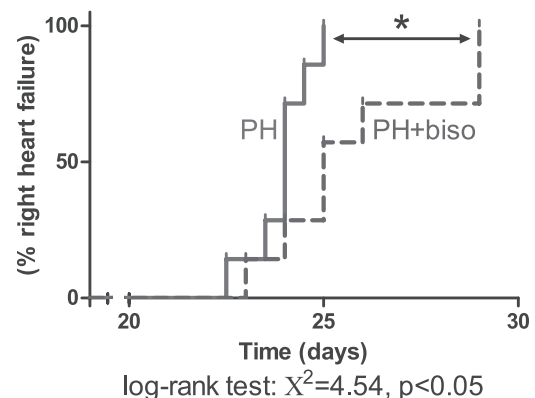


Figure 2. Bisoprolol treatment in PH significantly delayed time to manifest right heart failure. Log rank test: $X^2=4.54$; $P<0.05$. Control: $n=8$; PH/PH+biso: $n=7$. * indicates $P<0.05$ PH+biso versus PH.

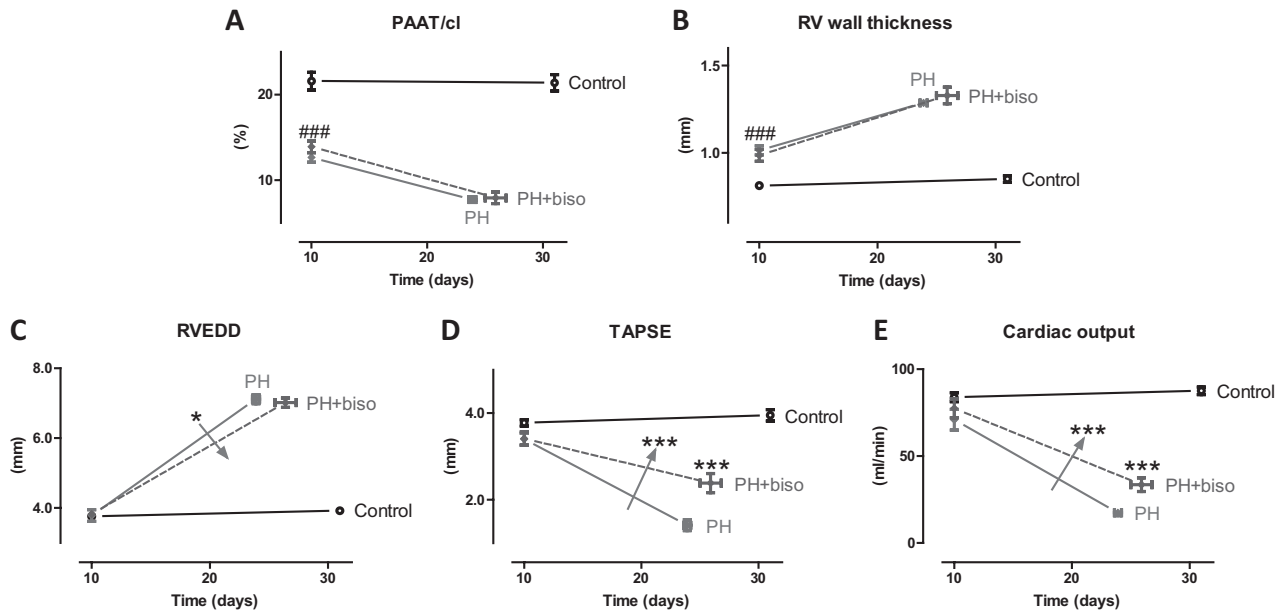


Figure 3. Disease progression during treatment period. Echocardiography could confirm the PH status at start of treatment (**A, B**: first time point, lower pulmonary artery acceleration time/cl and higher RV wall thickness). Bisoprolol treatment (PH+biso: dotted/blue) delayed RV dilatation (**C**, arrow) and reduced the decline in cardiac function (**D, E**: arrows), compared with vehicle-treated PH rats (PH: solid/red); numeric data are found in Table S1 (see the online-only supplement). In addition, at end of study cardiac function was better maintained in bisoprolol-treated PH rats. Data presented as mean \pm SEM, control: n=8; PH / PH+biso: n=7. ### indicates $P<0.001$ PH/PH+biso versus control; *, $P<0.05$, ***, $P<0.001$ PH+biso versus PH. PAAT/cl indicates pulmonary artery acceleration time normalized for cardiac cycle length (inversely correlated with RV systolic pressure); RVEDD, RV end-diastolic diameter; TAPSE, tricuspid annular plane systolic excursion.

versus PH+biso: $P<0.001$) and dP/dt_{max} end-diastolic volume (dP/dt_{max} -EDV) relation showed similar results (control: 3.7 ± 0.6 , PH: 11.1 ± 2.7 , PH+biso: 23.3 ± 5.7 mm Hg/ms/mL; PH versus PH+biso: $P<0.001$). Of note, after normalization of Ees for RV mass,²⁶ no significant difference was observed anymore between vehicle-treated PH rats and controls, whereas the difference in contractility between bisoprolol- and vehicle-treated PH rats remained statistically significant (Ees/RVmass, control: 40.3 ± 6.3 , PH: 43.6 ± 12.0 , PH+biso: 99.0 ± 10.9 mm Hg/mL/g; $P=0.02$ PH+biso versus PH). After normalization of Ees for RV volume, contractility was reduced significantly in vehicle-treated PH rats and improved toward normal value in the bisoprolol-treated PH rats (Ees/RVvolume, control: 2.14 ± 0.13 ; PH: 1.32 ± 0.08 ; PH+biso: 2.28 ± 0.14 mm Hg/mL² · 10³; PH versus PH+biso: $P<0.001$). Furthermore, bisoprolol treatment reduced RV end-diastolic pressures and Eed (measure of filling; Figures 4H and I).

Bisoprolol Reduced RV Fibrosis and RV Myocardial Inflammation

In line with previous echo findings, the right ventricles of PH rats at end of study were hypertrophied compared with controls (Figure 3B). No differences were observed between bisoprolol- and vehicle-treated PH rats, whether expressed as RV mass (irrespective of normalization), RV/(LV+S) ratio, or RV cardiomyocyte cross-sectional area (Tables S4 and S5; see the online-only supplement).

At start of treatment, no difference was observed between control and PH rats in RV capillary density or fibrosis, and there were no signs of cardiac inflammation (Table S2; see the online-only supplement). At end of study, the findings for

RV capillary density were similar; compared with control, capillary density was reduced in PH, without a significant difference between the 2 PH groups (Figure 5A, D, and G). More RV interstitial fibrosis was observed between PH rats and controls; interestingly, bisoprolol treatment significantly reduced RV fibrosis (Figure 5B,E, and H). Furthermore, the presence of (CD45⁺) inflammatory cells in RV myocardium of bisoprolol-treated PH rats was significantly less, compared with vehicle-treated PH rats (Figure 5C, F, and I). Leukocyte infiltration in the left ventricle was increased in bisoprolol- and vehicle-treated PH rats; however, these values were low and comparable with control values of the right ventricle (Table S5; see the online-only supplement). Autopsy and assessment of LV histology revealed no effect of bisoprolol (Tables S4 and S5; see the online-only supplement), compatible with a RV-specific effect of bisoprolol.

Bisoprolol Restored RV β -Adrenergic Receptor Signaling Pathway

Phosphorylation of both cMyBPC and cTnI (protein kinase A-mediated downstream targets of the β AR) were significantly higher in bisoprolol-treated PH rats in comparison with vehicle-treated PH rats (Figure 6).

Discussion

This study investigated the effects of bisoprolol treatment in experimental PH, focusing on RV function and remodeling. Using a comprehensive set of physiological and pathological end points, we have demonstrated that:

1. Chronic low-dosed bisoprolol treatment was well tolerated, and delayed time to manifest RHF.

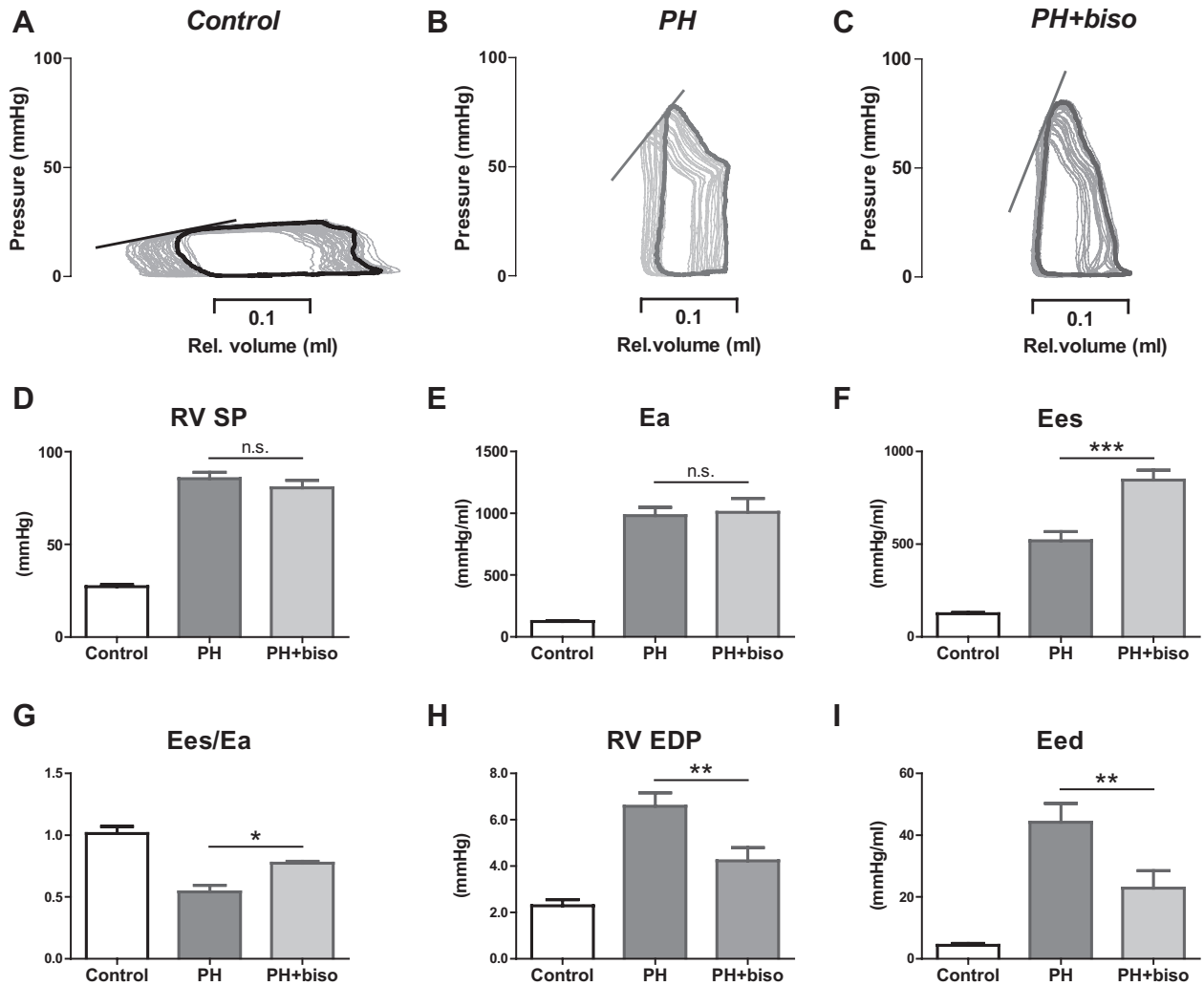


Figure 4. Pressure-volume analyses. Typical examples of the pressure-volume relation are shown for control, PH, and PH+biso (**A**, **B**, and **C**; line indicates end-systolic pressure-volume relationship). RV systolic pressures (RVSP) and arterial elastance (E_a ; measure of RV afterload) were increased equally in both PH groups (**D**, **E**). However, RV contractility (end-systolic elastance [E_{es}]) was increased significantly by bisoprolol treatment (**F**), resulting in partial normalization of ventriculo-arterial coupling (E_{es}/E_a ; **G**). Bisoprolol treatment also partially restored RV diastolic function, measured by RV end-diastolic pressure (RVEDP) and E_{ed} (**H**, **I**). Control: $n=8$, PH/PH+biso, $n=7$. * indicates $P<0.05$; **, $P<0.01$; ***, $P<0.001$; PH+biso versus PH.

- Bisoprolol treatment improved cardiac function by improving RV contractility (E_{es}), filling (E_{ed}), and ventriculo-arterial coupling (E_{es}/E_a).
- The cardiac-selective effects of bisoprolol can be attributed to restoration of RV β -adrenergic receptor signaling, the reduction of RV (interstitial) fibrosis, and RV myocardial inflammation.

These results suggest a potential role for β -blocker in PH that warrants further clinical investigation.

Bisoprolol Treatment Was Well Tolerated

Beta-blockers are currently not recommended, because PH patients are believed not to tolerate the acute (but transient) negative inotropic and chronotropic effects.^{1,13,14} To address this legitimate argument, we used an approach that was inspired by successful β -blocker use in LHF.

Low Versus High Dose

By definition, patients with LHF are hemodynamically compromised, and like in PH, their adrenergic system is overactivated as well.²⁷ To some extent, these 2 patient groups are therefore comparable.³ Interestingly, most LHF patients (approximately 85%) enrolled in clinical trials with β -blockers were able to tolerate short- and long-term treatments with this drug, and reached the maximum planned target dose, when β -blockers are introduced at a very low dose, followed by gradual dosage increase (“start low, go slow”).¹¹ In addition, whereas the adverse effects of β -blockers are dose dependent, the beneficial effects are associated with heart rate reduction, which can be achieved by lower dosages.²⁸ In this study, we used the minimum dose that effectively blunted heart rate response. This was accompanied by only minimal side effects, and was therefore well tolerated by the PH rats (minor effect on blood pressure, no effect on activity). Compared with other rat studies that used biso-

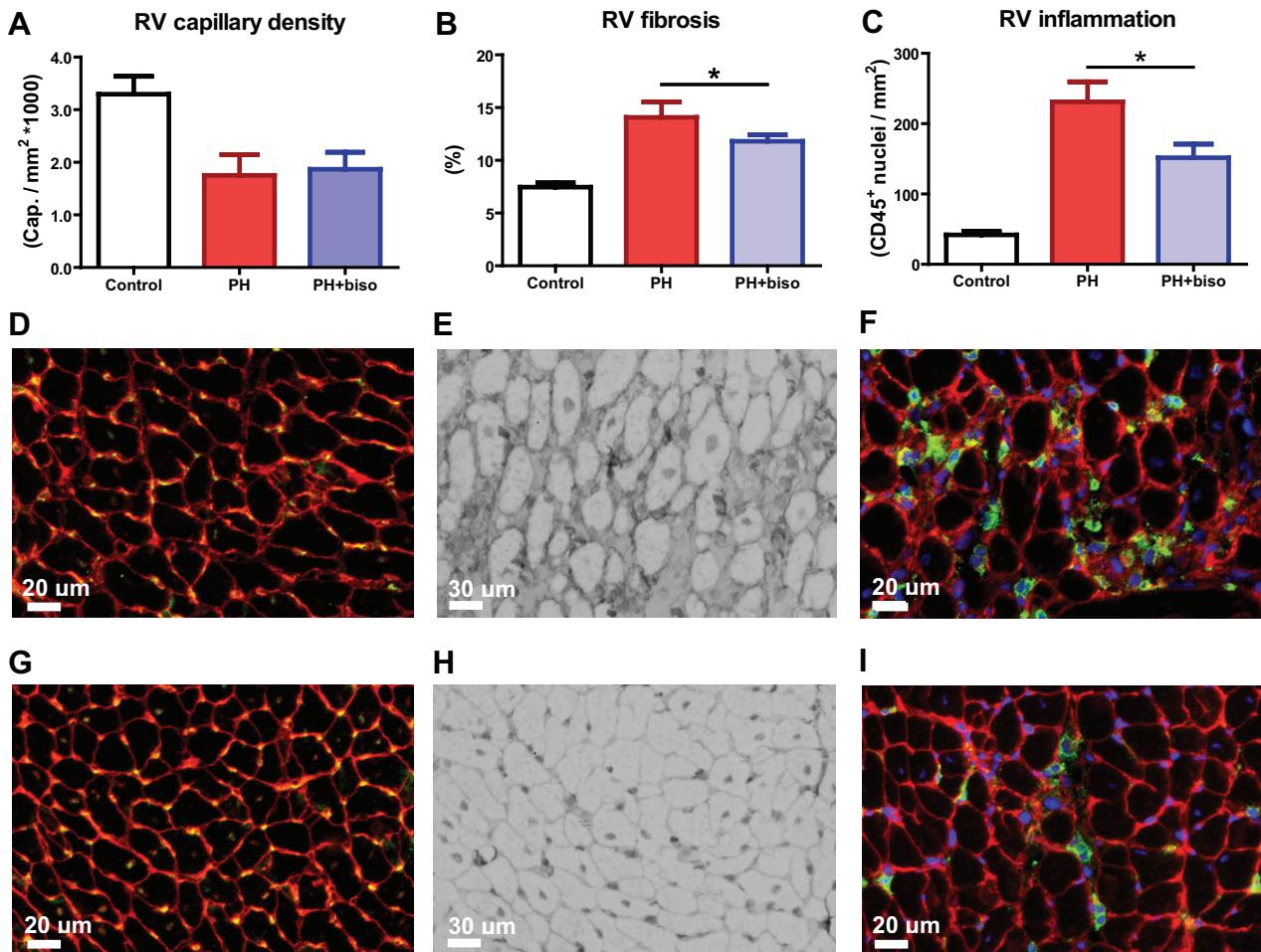


Figure 5. Bisoprolol treatment reduced RV fibrosis and RV myocardial inflammation. Histomorphometric analyses revealed significant and selective reduction of RV interstitial fibrosis and RV myocardial inflammation in bisoprolol-treated PH rats (**B**, **C**). No difference was observed for RV capillary density (**A**). Typical examples are shown of histological sections of the right ventricle of vehicle- (PH: **D**, **E**, and **F**) and bisoprolol-treated PH rats (PH+biso: **G**, **H**, and **I**), stained for RV capillarization (**D**, **G**: endothelium marker CD31 is stained green, cell membranes red; capillaries appear as small yellow/orange dots), fibrosis (**E**, **H**: picrosirius red staining, dark gray), and infiltrating inflammatory cells (**F**, **I**: lymphocyte marker CD45 is stained green, cell membranes red, nuclei blue). Control: n=8; PH/PH+biso: n=7. *indicates $P < 0.05$ PH+biso versus PH.

prolol (typically 60 mg/kg), the dose used in this study can be considered low.²⁹

Hemodynamic data at day 10 suggests limited functional hemodynamic compromise of the PH rats at start of treatment. Unfortunately, the progressiveness of the model did not permit starting treatment at a later time point. However, like in LHF,¹¹ β -blocker use most likely will be limited to PH patients with some cardiac reserve, in order to cope with its acute negative inotropic effects.

Selective Versus Unselective β -Blocker

Of all β -blockers, only bisoprolol, carvedilol, and metoprolol have been proven to reduce mortality in LHF.¹¹ Of these 3, bisoprolol is the most β_1 -cardioselective.¹⁰ We chose bisoprolol to avoid potential harmful effects of β_2 -mediated blockade. The β_2 -subtype is the predominant β AR present in the pulmonary vasculature. Blockade of the β_2 -receptors may lead to smooth muscle contraction, which could result in a further increase in pulmonary vascular resistance and RV pressures. Nevertheless, the clinical relevance of β_1 -selectivity in PH is unknown.

Beneficial Effects of Bisoprolol

To ease the clinical interpretation of our findings, we used robust and clinically relevant outcome measures to investigate the effects of bisoprolol. We explicitly evaluated pressure-volume relations, because it is considered the gold standard to describe cardiac function,^{20,26} and more specifically, to address the potential risk of ventriculo-arterial uncoupling after β -blocker use in PH, as raised by others.¹³ In contrast to what was feared, we observed partial normalization of the ventriculo-arterial coupling, which may be explained by chronic opposed to acute drug administration.

Measurements of RV wall thickness and diastolic diameter at end of study protocol failed to detect changes that could explain the beneficial effects of bisoprolol. However, it should be noted that these measurements were obtained at a stage of terminal right heart failure, which was reached at a later time point in the bisoprolol-treated PH group than the PH control group: eg, the progression of RV dilatation was significantly less in the bisoprolol-treated rats (Figure 3C: RV end-diastolic diameter; Table S3 [see the online-only supplement]). Hence, the beneficial effect of bisoprolol between

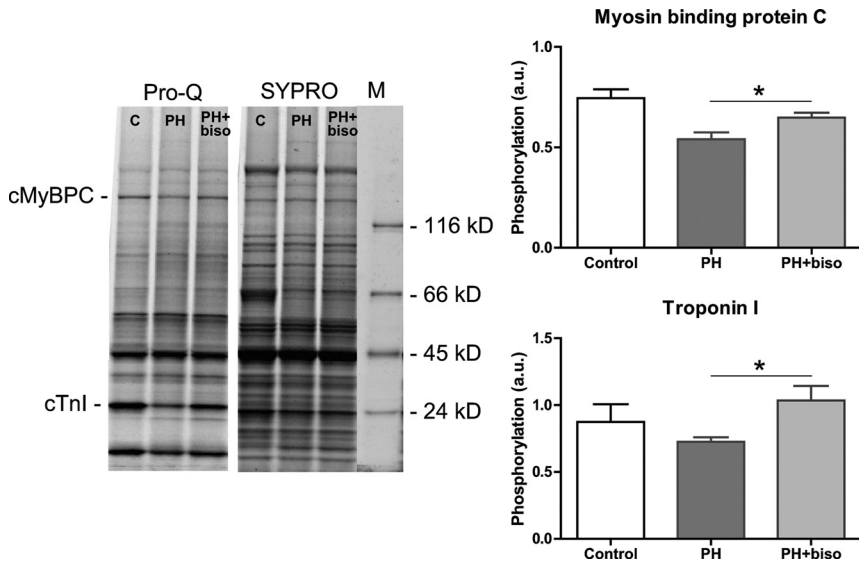


Figure 6. Bisoprolol treatment restored β -adrenergic receptor signaling. A typical example of a pro-Q/SYPRO gel is shown for control, vehicle-treated, and bisoprolol-treated PH rats. Analyses revealed increased phosphorylation of downstream β AR-targets myosin binding protein C (cMyBPC) and troponin I (cTnI) after bisoprolol treatment. Control: n=8; PH/PH+biso: n=7. * indicates $P < 0.05$ PH+biso versus PH.

both groups are reflected more by the time elapsed to reach right heart failure than the hemodynamic findings at right heart failure. In addition, we observed less RV inflammation and fibrosis, together with an increase in RV contractility normalized for hypertrophy (Ees/RVmass), in the bisoprolol-treated PH group compared with the control PH group. This implies that bisoprolol treatment results in improved intrinsic properties of RV cardiomyocytes that cannot be detected by relatively crude measures such as RV wall thickness and diastolic diameters.

Only a few studies have evaluated the (chronic) effects of β -blockers in the context of PH.¹⁴ A recent paper by Bogaard et al¹⁶ provides some hemodynamic and molecular insights in the effects of carvedilol (a nonspecific $\alpha_1/\beta_1/\beta_2$ -adrenergic receptor blocker) on echocardiographic parameters and molecular analyses in PH rats. For the first time, we demonstrate that the positive effects of β -blocker treatment are RV-specific and could delay disease progression, improve RV function, and (partially) restore ventriculo-arterial coupling and β AR-signaling. Bogaard et al¹⁶ also observed less RV fibrosis after β -blocker treatment. They found an increase in RV capillary density, which we could not confirm. We, on the other hand, observed a reduction in RV inflammation, which was not studied in detail by Bogaard et al.¹⁶ Differential effects of the β -blockers used might explain these subtle differences. An alternative explanation is related to differences in disease severity of the experimental models used; in our monocrotaline model, all rats developed right heart failure within 4 weeks, whereas all hypoxia/Sugen rats survived 8 weeks after induction of PH. As a consequence, treatment duration in our monocrotaline model was relatively short (median treatment was 2.5 weeks) compared with Bogaard's Sugen/hypoxia model (4 weeks).¹⁶ It is possible that bisoprolol treatment reduced RV inflammation and RV fibrosis (early effects), but that its treatment duration was insufficient to enhance RV capillary density (late effect). Usui et al³⁰ also investigated the effect of carvedilol in monocrotaline-treated rats. They too observed survival benefit with β -blocker, but unfortunately they did not report any measures on cardiac

function, and focused mainly on LV rather than RV remodeling. Also, no information was provided on possible side effects and tolerability. In addition, Ishikawa et al³¹ reported beneficial effects of arotinolol (a nonspecific β -blocker) using the same PH rat model. However, the clinical implications of this study are limited; arotinolol was studied to prevent rather than to treat PH-associated RHF, and, unlike bisoprolol, arotinolol is not clinically used or Food and Drug Administration approved.

Potential Mechanisms

In this proof of concept study, we did not perform in-depth analysis on cellular and molecular effects of bisoprolol. Nonetheless, our histological and protein analysis may provide some mechanistic insights, based on the experiences with β -blockers in LHF.

Although the β -adrenergic system in LHF is incompletely understood, it is generally believed to exert its beneficial effects by blunting of the sympathetic overactivity, resulting in reduced desensitization of the β AR and restoration of β AR-signaling.³² Protein kinase A is a central player in the β AR-signaling.³³ Therefore, we investigated the effect of bisoprolol on phosphorylation of contractile proteins cMyBPC and cTnI, 2 main targets of protein kinase A. Interestingly, we observed that bisoprolol restored phosphorylation of both cMyBPC and cTnI, compared with vehicle treatment. This suggests that bisoprolol restored RV cardiomyocyte β AR-signaling.

An alternative explanation for the observed beneficial effects of bisoprolol therapy might be that a reduction in heart rate by β -blockers could have prevented sustained high levels of RV wall stress. We previously observed that exercise increased myocardial inflammation in experimental PH, and related this to increased RV wall stress during episodes of activity,⁹ comparable with what has been described in detail by Sun et al.³⁴ This is in agreement with the observation of the present study that bisoprolol prevents rather than reverses RV inflammation, as no inflammation was observed at start of treatment, which also argues against direct cardiac inflammatory effects of monocrotaline.

Clinical Relevance

We demonstrated that β -blocker therapy is beneficial in experimental PH. However, the monocrotaline model exhibits alterations in the β -adrenergic system that resemble those in human PH; others have previously demonstrated that in monocrotaline-treated rats with RHF, heart rate variability is reduced,³⁵ plasma norepinephrine levels are increased, and β_1 -adrenergic receptor density of the right ventricle is decreased,³⁶ similar to clinical PH.³

Despite bisoprolol treatment all PH rats eventually developed right heart failure. However, we want to emphasize that the effects of bisoprolol were achieved in the absence of “traditional” vasodilating therapies (eg, bosentan) that modulate the progression of pulmonary vascular remodeling. The findings of this study therefore provide a rationale to investigate the role of (cardioselective) β -blockers as an add-on therapy in the management of clinical PH.

Conclusions

In our PH rat model, bisoprolol treatment was well tolerated and beneficial. It delayed the progression toward RHF, which was attributed to improved RV contractility and compliance, and was accompanied by restored β -adrenergic receptor signaling and reduced RV fibrosis and inflammation. Future studies are necessary to address the clinical implications of our findings.

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Disclosures

None.

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CLINICAL PERSPECTIVE

The use of beta-blockers is presently contraindicated in the clinical management of patients with pulmonary arterial hypertension (PH). Increased sympathetic activity is believed to be a compensatory mechanism to maintain cardiac output by increasing heart rate and right ventricular (RV) contractility. However, as has been shown for left heart failure, chronic adrenergic overstimulation might be detrimental in the long run. In the present study, we therefore studied the chronic effects of a cardio-selective β -blocker (bisoprolol) on disease progression, RV function, and β -adrenergic signaling. In our PH rat model, bisoprolol treatment was well tolerated and beneficial; it delayed the progression towards right heart failure, which was attributed to improved RV contractility and compliance, and was accompanied by restored β -adrenergic receptor signaling and reduced RV fibrosis and inflammation. These findings provide a rationale to investigate the clinical application of β -blockers in PAH. However, the acute negative inotropic effects of β -blockers on the pressure overloaded right ventricle necessitates a very careful approach. For that reason, we are currently conducting a prospective clinical study to evaluate whether a cautious introduction of β -blockers in New York Heart Association II/III PAH patients is tolerated and safe.