Hypertension

Opposite Effects of Training in Rats With Stable and Progressive Pulmonary Hypertension

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Background—Exercise training in pulmonary arterial hypertension (PH) is a promising adjunct to medical treatment. However, it is still unclear whether training is beneficial for all PH patients. We hypothesized that right ventricular adaptation plays a pivotal role in the response to training.

Methods and Results—Two different dosages of monocrotaline were used in rats to model stable PH with preserved cardiac output and progressive PH developing right heart failure. Two weeks after injection, PH was confirmed by echocardiography, and treadmill training was initiated. Rats were trained for 4 weeks unless manifest right heart failure developed earlier. At the end of the study protocol, all rats were functionally assessed by endurance testing, echocardiography, and invasive pressure measurements. Lungs and hearts were further analyzed in quantitative histomorphologic analyses. In stable PH, exercise training was well tolerated and markedly increased exercise endurance (from 25 ± 3.9 to 62 ± 3.9 minutes; $P<0.001$). Moreover, capillary density increased significantly (from 1.21 ± 0.12 to 1.51 ± 0.07 capillaries per cardiomyocyte; $P<0.05$). However, in progressive PH, exercise training worsened survival (hazard ratio, 2.7; 95% confidence interval, 1.1 to 14.2) and increased pulmonary vascular remodeling. In addition, training induced widespread leukocyte infiltration into the right ventricle (from 135 ± 14 to 276 ± 18 leukocytes per 1 mm^2; $P<0.001$).

Conclusions—In our rat model, exercise training was found to be beneficial in stable PH but detrimental in progressive PH. Future studies are necessary to address the clinical implications of our findings. (***Circulation***. 2009;120:42-49.)

Key Words: capillaries ∎ exercise ∎ hypertension, pulmonary ∎ inflammation ∎ pulmonary heart disease

Pulmonary arterial hypertension (PH) is characterized by progressive pulmonary vascular remodeling, which importantly increases right ventricular (RV) afterload, eventually leading to right heart failure and premature death.[1] Traditionally, PH patients were advised to limit physical activity because of risk of fatal cardiovascular compromise.[2]

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Recent developments, however, have challenged this view.[3] First, the prognosis of PH patients improved with the introduction of various potent PH-specific medications in the last decades.[4] Second, several studies have demonstrated beneficial effects of training in patients with chronic obstructive pulmonary disease and with congestive heart failure, and training was beneficial even for the most severely affected patients (GOLD IV, New York Heart Association class IV) often suffering from secondary PH.[5,6] Finally, in a recent clinical trial of 30 stable PH patients under optimized medical

treatment, Mereles et al[7] reported marked improvement in exercise capacity and quality of life after exercise training.

Although training might be a promising adjunct to medical treatment in PH, it remains to be elucidated whether exercise training is beneficial for all PH patients and what its effect is on RV function and remodeling. RV adaptation might be a discriminating factor for responsiveness to training. During exercise, pulmonary artery pressures and RV afterload increase,[8] resulting in a transient elevation of RV wall stress. Although this is unknown for right heart failure, for left heart failure, it has been demonstrated that even a temporary elevation of wall stress can upregulate local proinflammatory factors, leading to leukocyte infiltration into the myocardium.[9] We hypothesized that such a proinflammatory reaction in the right ventricle might outweigh the positive effects of exercise training, especially in the presence of RV maladaptation to pressure overload.

We therefore conducted an experimental study and assessed the effects of exercise training in 2 phenotypes of PH,
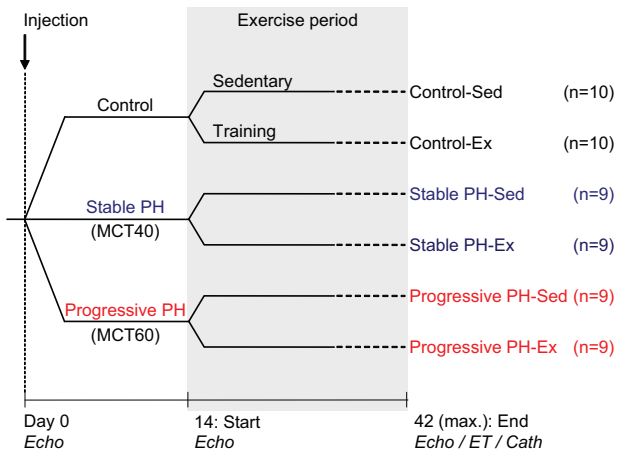
Figure 1. Study design. The effect of exercise training was studied in 2 distinct phenotypes of established PH (stable and progressive PH). Start indicates the start of the exercise period (14 days after injection); End, end of the study protocol (when manifest signs of right heart failure developed or 42 days after monocrotaline injection); Echo, echocardiographic evaluation; ET, endurance testing; and Cath, RV catheterization.

namely stable PH with a preserved cardiac output (CO) at rest and progressive PH developing right heart failure. Using a comprehensive set of physiological and pathological end points, we documented beneficial effects in stable PH but detrimental effects in progressive PH.

Methods

All experiments were approved by the institutional animal care and use committee at the VU University.

Experimental PH

Male Wistar rats were used (56 in total; weight, 150 to 175 g; Harlan, Horst, the Netherlands). PH was induced by a single subcutaneous injection of monocrotaline (Sigma-Aldrich, Zwijndrecht, the Netherlands) dissolved in sterile saline.

Monocrotaline 60 mg/kg body mass was used to model progressive PH developing right heart failure (n=18); with a dose of 40 mg/kg monocrotaline, stable PH with a preserved CO was mimicked (n=18).[10,11] The control group was injected with saline only (n=20).

Study Design and Training Program

The exercise program was adopted from a validated exercise program for Wistar rats described by Fenning et al.[12] In the first week, all rats were accustomed to treadmill running; mild electric stimulation was used to encourage the rats to run. Then, rats were randomly assigned to any of the 3 experimental groups (control, stable PH, or progressive PH) and injected accordingly with monocrotaline or saline (Figure 1). In the next 2 weeks, all rats were placed on the treadmill for 1 minute a day (5 times a week at a constant speed of 13.3 m/min with no slope). After these 2 weeks, the rats were again randomly assigned to an exercise training program (control-Ex, stable PH-Ex, or progressive PH-Ex; 5 times a week for 30 minutes at 13.3 m/min with no slope) or the sedentary group (control-Sed, stable PH-Sed, or progressive PH-Sed; 5 times a week for 1 minute at 13.3 m/min with no slope). The level of training represented moderate exercise intensity (≈50% $\dot{V}O_2$max).[13] Animals were trained for a maximum of 4 weeks (from day 14 until day 42 after monocrotaline injection). Rats that developed manifest clinical signs of right heart failure (defined as >5% loss of body mass a day and/or respiratory distress, cyanosis, lethargy) were euthanized early, in keeping with the protocol approved by the institutional animal care committee. Manifest right heart failure was the survival end point and recorded as an event in the survival analysis.[14]

Endurance Test

Endurance testing was performed only on rats that completed the 4-week exercise program.[12] The treadmill was set at a constant speed of 15 m/min and a slope of 20°. The time from start until exhaustion was used as a measure for the exercise endurance of the rats. Exhaustion was established when the rats accepted the electric stimulus 3 consecutive times as opposed to running. The maximal running time was 90 minutes, which was achieved by all control rats.

Hemodynamic Evaluation

Echocardiography

The rats were evaluated by echocardiography at baseline (just before they received their injection), at the start of training, and at the end of the study protocol (when manifest signs of right heart failure developed or 42 days after monocrotaline injection). Transthoracic echocardiographic measurements (ProSound SSD-4000 system equipped with a 13-MHz linear transducer [UST-5542], Aloka, Tokyo, Japan) were performed on anesthetized but spontaneously breathing rats (isoflurane 2.0% in 1:1 O₂/air mix; Pharmachemie, Haarlem, the Netherlands) as described previously.[15]

Analyses were performed offline (Image-Arena 2.9.1, TomTec Imaging Systems, Unterschleissheim/Munich, Germany). Measured parameters for cardiac and RV function were Doppler-derived stroke volume, CO, and tricuspid annular plane systolic excursion (TAPSE). Parameters for RV remodeling were RV end-diastolic diameter and RV wall thickness. Parameters for pulmonary vascular remodeling were pulmonary artery acceleration time normalized for cycle length and pulmonary vascular resistance (PVR).

Disease progression of PH during the period of exercise training was expressed as percentage changes in hemodynamics over time, ie, change in CO: $\Delta CO=[(CO_{end\ of\ protocol}-CO_{start\ of\ training})/CO_{start\ of\ training}]\times100\%/days$ of training. Other parameters for disease progression (change in stroke volume, ΔTAPSE, etc) were calculated similarly. The noninvasive estimations of RV systolic pressures, PVR, and RV wall stress are described in the online-only Data Supplement.

Invasive RV Pressure Measurements

At the end of the study protocol, open-chest RV catheterization was performed under general anesthesia in all animals (isoflurane 2.0% in 1:1 O₂/air mix) as described elsewhere.[16] Before the procedure, the rats were intubated (16-gauge Teflon tube) and attached to a mechanical ventilator (Micro-Ventilator, UNO, Zevenaar, the Netherlands; ventilator settings: breathing frequency, 80 breaths per minute; pressures, 9/0 cm H₂O; inspiratory/expiratory ratio, 1:1). The right ventricle was approached via a lateral right thoracotomy through the fifth intercostal space. RV pressures were recorded by the use of a high-fidelity catheter tip transducer (Mikro-Tip SPR-671, Millar Instruments, Houston, Tex). Analyses were performed when steady state was reached over an interval of at least 10 seconds and averaged.

Histology

After the final hemodynamic assessment, the rats were euthanized by exsanguination (under isoflurane), and heart, lungs, and other major organs were harvested. Lungs were weighed, and the airways of the left lobe were subsequently filled with a 1:1 mixture of saline and cryofixative (Tissue-Tek optical coherence tomography compound, Sakura Fintek Europe, Zoeterwolde, the Netherlands) and snap-frozen in liquid nitrogen. The right lobe was used to measure the ratio of dry to wet lung mass. The heart was perfused, weighed, dissected, and snap-frozen in liquid nitrogen.

Histomorphometric Analysis of Heart and Lungs

The determination of cardiomyocyte cross-sectional area, cardiac fibrosis, and relative wall thickness of pulmonary arterioles (PAs) is described in detail in the online-only Data Supplement.

Analysis of capillary density and cardiac inflammation was performed with quantitative immunofluorescence microscopy. Briefly, cardiac cryosections (5 μm) were incubated for 60 minutes with primary CD31 (1:35; sc-1506-R, Santa Cruz Biotechnology,

Santa Cruz, Calif) and CD45 (1:25; sc-53045, Santa Cruz Biotechnology) antibodies for capillary density and leukocyte infiltrations, respectively, followed by appropriate secondary antibody staining and WGA (glycocalyx) and DAPI (nuclei) counterstaining. Image acquisition was performed on a Marianas digital imaging microscopy workstation (Intelligent Imaging Innovations, Denver, Colo). SlideBook imaging analysis software (SlideBook 4.2, Intelligent Imaging Innovations) was used to quantify the images semiautomatically. Capillary density was expressed as the number of capillaries per cardiomyocyte or number of capillaries per section area, measured in at least 3 randomly chosen areas per ventricle, where cardiomyocytes were transversally sectioned.[17] Leukocyte infiltration was expressed as the number of positive CD45 nuclei per section area, measured over at least 3 randomly chosen areas per ventricle.[18]

Statistical Analysis

All analyses were performed in a blinded fashion. All data were verified for normal distribution. Data are presented as mean±SEM, and analyses were performed on all rats unless stated otherwise. A value of $P<0.05$ was considered significant.

Survival estimates were performed by Kaplan–Meier analyses, with posthoc comparisons performed by the log-rank test. Hazard ratios were calculated by the proportional-hazards model. For all other in vivo data, 2-way ANOVA was used. The interaction between PH status and training status was tested; subsequently, Bonferroni posthoc tests were performed (training versus sedentary in the 3 experimental groups). All reported probability values of posthoc comparisons are Bonferroni corrected (SPSS 16.0 for Windows, SPSS, Chicago Ill). For histological data, multilevel analysis was used to correct for the nonindependence of successive measurements of cross-sectional areas and PA wall thickness per animal (MLwiN 2.02.03, Center for Multilevel Modeling, Bristol, UK).[19]

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Established PH at Start of Training

Estimated RV systolic pressure (using pulmonary artery acceleration time normalized for cycle length) at the start of training was elevated for stable PH and progressive PH compared with control (estimated RV systolic pressure: stable PH, 36±2.8 mm Hg; progressive PH, 48±3.5 mm Hg; control, 26±2.3 mm Hg; $P<0.001$; Table I of the online-only Data Supplement). In addition, compared with control, PVR for stable and progressive PH was higher. Together with the rise in pulmonary pressures, a modest increase in RV wall thickness was found, indicating mild RV hypertrophy at the start of training. At this time point, there were no signs of cardiac dysfunction or adverse remodeling measured by CO, stroke volume, heart rate, TAPSE, or RV end-diastolic diameter (Table I of the online-only Data Supplement).

Induction of Stable Versus Progressive PH by Different Monocrotaline Dosages

Serial echocardiographic measurements revealed different phenotypes of PH induced by monocrotaline 40 or 60 mg/kg (Figure I of the online-only Data Supplement). In stable PH-Sed (40 mg/kg monocrotaline, untrained rats), from day 14 after monocrotaline injection, there was no significant increase in PVR, and resting CO was preserved (ΔPVR= 7.5±5.0%/d; ΔCO=−0.74±0.81%/d; CO at end=109±10 mL/min). Nevertheless, some signs of RV dysfunction and adverse remodeling were observed in stable PH-Sed at the end
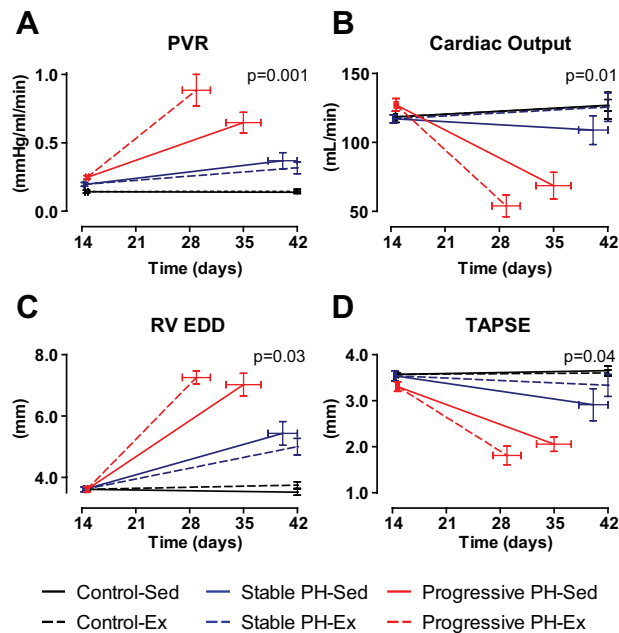

Figure 2. Opposite effects of training in stable vs progressive PH were found for all important hemodynamic parameters for disease progression (indicated by the slope of the connecting lines from the start of training to the end of the study protocol). All data are presented as mean±SEM. *P* values represent the interactive effect per individual parameter. Each line corresponds with an experimental group (see bottom). Numerical data at the start of training and on disease progression can be found in Tables I and II of the online-only Data Supplement. EDD indicates end-diastolic diameter.

of the study protocol (TAPSE: 2.9±0.3 mm for stable PH-Sed versus 3.7±0.1 mm for control-Sed; RV end-diastolic diameter: 5.4±0.4 mm for stable PH-Sed versus 3.5±0.1 mm for control-Sed; all $P<0.05$). In progressive PH-Sed (60 mg/kg monocrotaline, untrained rats), a rapid increase in PVR and a marked decline in CO were seen (ΔPVR=11±4.1%/d; ΔCO=−3.1± 0.76%/d; CO at end=69±9.7 mL/min; all $P<0.05$ versus stable PH-Sed).

Effects of Training on Disease Progression in Stable Versus Progressive PH

Serial echocardiographic measurements were used to study the effect of training on disease progression in control, stable PH, and progressive PH. Figure 2 shows the evolution of the various echocardiography-derived hemodynamic parameters over time for the different experimental groups (for numerical data, see Table II of the online-only Data Supplement). The divergent effect of training on disease progression is especially evident for CO (Figure 2B). During the exercise period, a steeper fall in CO was observed for progressive PH-Ex compared with progressive PH-Sed, whereas CO was better preserved in stable PH-Ex than stable PH-Sed.

Effects of Training on Survival and Endurance in Stable Versus Progressive PH

The loss of body mass and decline in CO were closely correlated ($r=0.74$, $P<0.001$), which verifies the clinical criteria used for right heart failure. From these criteria, we observed that exercise training decreased survival signifi-
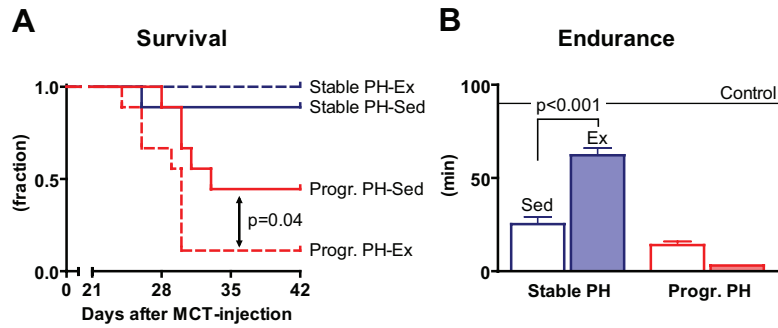
Figure 3. A, Training had a detrimental effect on survival in progressive PH, whereas it did not affect survival in stable PH. All rats from the control groups survived and are omitted here for clarity. B, Training improved endurance >2-fold in stable PH, whereas an opposite trend was observed in progressive PH (interactive effect, $P<0.001$). All rats in the control groups reached the predefined maximum endurance time (90 minutes; horizontal line). All data are presented as mean±SEM. Endurance testing was performed only on surviving rats, therefore n=10 (control-Sed), n=10 (control-Ex), n=8 (stable PH-Sed), n=9 (stable PH-Ex), n=4 (progressive PH-Sed), and n=1 (progressive PH-Ex). MCT indicates monocrotaline.

cantly in progressive PH (hazard ratio: progressive PH-Ex versus progressive PH-Sed, 2.7; 95% confidence interval, 1.1 to 14.2). In stable PH-Sed, 1 rat prematurely developed right heart failure; all rats in the stable PH-Ex and control groups survived (Figure 3A).

At 4 weeks, training significantly improved exercise endurance by 2-fold in stable PH (endurance: 62±3.9 minutes for stable PH-Ex versus 25±3.9 minutes for stable PH-Sed; $P<0.001$), whereas the opposite trend was observed in progressive PH. A strong significant interaction between training status and PH status was present ($P<0.001$; Figure 3B). The opposite effects of training on survival and endurance are in line with previous findings on disease progression.

RV Catheterization at the End of the Study Protocol

RV catheterization at the end of the study protocol confirmed the PH status of monocrotaline-treated rats (Figure II of the online-only Data Supplement). There was a strong monocrotaline dose-dependent response at the end of the study protocol ($P<0.001$); RV systolic pressure at rest doubled in stable PH and almost tripled in progressive PH compared with control, and a similar pattern was seen for RV diastolic pressures. In addition, the intrinsic RV contractility and relaxation were significantly changed in stable and progressive PH compared with control. However, no (statistical) differences in RV dP/dtmax and RV dP/dtmin were found between the 2 PH phenotypes. RV catheterization at the end of the study protocol revealed no (interactive) effect of training.

Effects of Training on Pulmonary Vascular Remodeling in Stable Versus Progressive PH

Training increased (wet) lung weights in progressive PH ($P<0.001$), whereas it had no effect on lung weights in stable PH, independently of normalization (ie, normalization by body mass or tibia length; Figure 4A, Table III of the online-only Data Supplement). Moreover, a strong interaction between training status and PH status was present ($P<0.001$). Ratios of wet to dry lung mass were similar for all experimental groups (ratios of wet to dry lung mass between groups varied from 4.9±0.1 to 5.2±0.1; see Table III of the online-only Data Supplement). This suggests that training modulated pulmonary vascular

remodeling and that the observed differences are not likely to be attributed to pulmonary edema. Measurements of PA wall thickness confirmed the interactive effect of training on pulmonary vascular remodeling (Figure 4B). Echocardiography-derived PVR at the end of the study protocol and PA wall thickness correlated well ($r=0.82$, $P<0.001$), indicating that the pulmonary vascular remodeling must have had profound hemodynamic effects in vivo.

Effects of Training on Cardiac Remodeling in Stable Versus Progressive PH

Histomorphology

In contrast with pulmonary vascular remodeling, the amount of RV hypertrophy was similar among stable and progressive PH, whether it was expressed by RV mass (normalized or not) or RV/(LV+S), where LV is left ventricle and S is
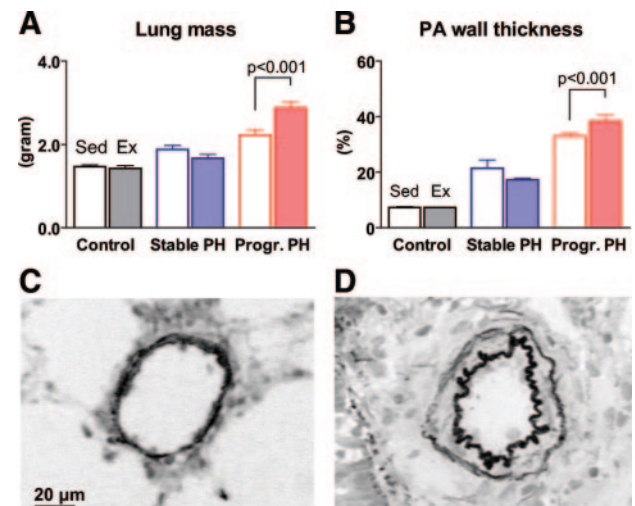

Figure 4. Training significantly worsened pulmonary vascular remodeling in progressive PH, demonstrated by elevated (wet) lung mass and increased PA wall thickness in progressive PH-Ex vs progressive PH-Sed ($P<0.001$). Moreover, significant interactions between training status and PH status were present (A and B; $P<0.001$ for lung mass, $P<0.01$ for PA wall thickness). Bottom, Typical examples of PAs (elastica von Gieson, ×200 magnification) of control-Sed (C) and progressive PH-Ex (D). Data are presented as mean±SEM.
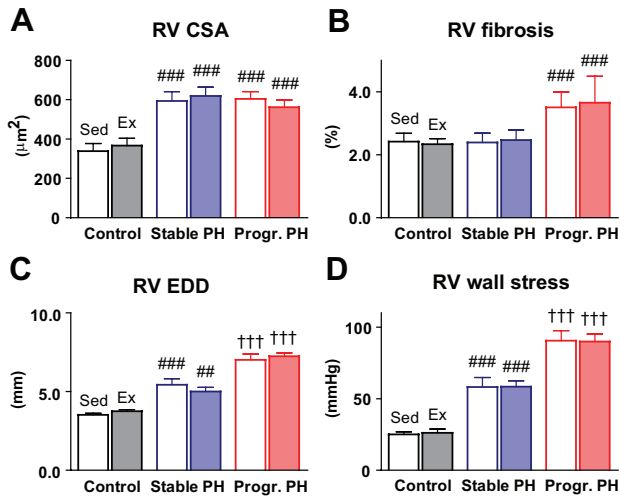
Figure 5. A, The amount of RV hypertrophy indicated by RV cross-sectional area (CSA) was similar in stable and progressive PH. B, RV fibrosis was seen only in progressive PH, and no (interactive) effect of training was observed. The largest RV diameters were observed in progressive PH (C), translating into the highest RV wall stress (D). All data are presented as mean±SEM. EDD indicates end-diastolic diameter. ##$P < 0.01$, ###$P < 0.001$ vs control; †††$P < 0.001$ vs stable PH ($P < 0.001$ vs control).

Figure 6. Training improved RV capillarization in stable PH, whereas a trend for an opposite effect was observed in progressive PH (capillarization expressed as ratio of capillaries to cardiomyocytes [A; Cp/Cm] or capillaries per area [B]). Moreover, a significant interaction between training status and PH status was present ($P < 0.01$). Bottom, Typical examples of RV capillarization (immunofluorescence CD31, ×100 magnification) of control-Sed (C) and stable PH-Ex (D). Green indicates CD31; red, cardiomyocyte cell membranes. Capillaries can be identified as green/yellow dots (merging of red and green; arrow). All data are presented as mean±SEM.

septum (Table III of the online-only Data Supplement), confirming echocardiographic observations. Measurements of RV cardiomyocyte cross-sectional area also showed similar RV hypertrophy between stable and progressive PH compared with control (Figure 5A). RV fibrosis was significantly increased in progressive PH only; no additional effect of training was found (Figure 5B). Similar levels of RV hypertrophy but differences in RV diameters (Figure 5C) indicate concentric instead of eccentric remodeling, which translated to severely elevated RV wall stress levels for progressive PH but only moderately elevated wall stress levels for stable PH; no interactive effect of training was observed (Figure 5D).

Analyses of LV myocardium revealed atrophy (evident from lower LV mass and lower LV cardiomyocyte cross-sectional area) and increased fibrosis in progressive PH only. No effect of training was observed on LV myocardium in progressive PH (Figure III of the online-only Data Supplement).

Cardiac Capillarization and Inflammation

Training improved RV capillarization in stable PH by ≈25%, whereas a trend for the opposite effect was seen in progressive PH whether expressed as a ratio of capillaries per cardiomyocyte or as capillaries per section area (Figure 6A and 6B). Moreover, a significant interaction between training status and PH status was present ($P < 0.01$). Training had no effect on capillarization in the LV (Figure IIID of the online-only Data Supplement).

In progressive PH, clusters of leukocytes were observed in various parts of the RV myocardium (Figure 7D). As a result, the number of leukocytes was significantly higher in progressive PH compared with control. Training dramatically increased the RV leukocyte infiltration in progressive PH, whereas in stable PH, it remained unchanged (Figure 7A).
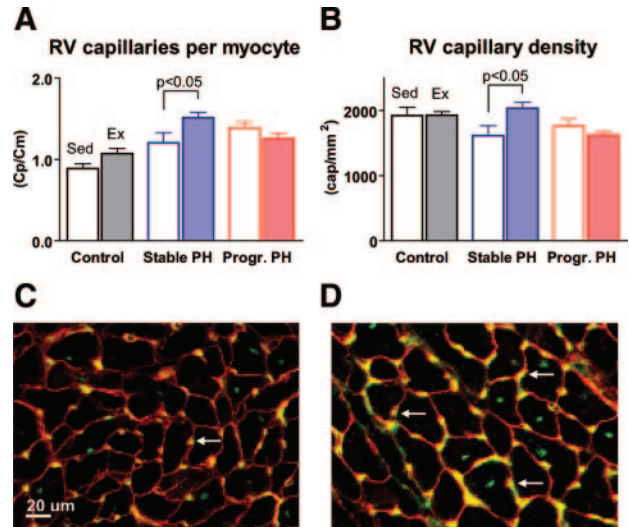
Moreover, a very significant interaction between training status and PH status was present ($P < 0.001$). Leukocyte infiltration was observed only in the RV. Analysis of the LV showed no differences between groups; their values were even slightly lower than RV control values (Figure 7B).

Discussion

To the best of our knowledge, this is the first study to investigate the effects of training in stable and progressive PH, focusing on RV function and remodeling. Using a comprehensive set of physiological and pathological end points, we have demonstrated that exercise training was well tolerated and beneficial in PH with a preserved CO. In this group, training had no adverse effects on disease progression; it improved endurance and was associated with enhanced RV capillarization. However, exercise training was detrimental in progressive PH developing right heart failure. In this situation, exercise had adverse effects on hemodynamics and accelerated the progression to right heart failure. Moreover, exercise was associated with adverse pulmonary vascular remodeling and massive RV inflammation.

Functional Improvement After Training Associated With Enhanced Capillarization

The only prospective clinical trial on exercise training in PH, by Mereles et al,[7] showed that functional capacity and quality of life of stable PH patients could be improved markedly after training. The general hemodynamic characteristics of the subjects in that trial were similar to the stable PH group in our study; comparable pulmonary artery pressures were found, together with a mildly depressed cardiac index at rest and
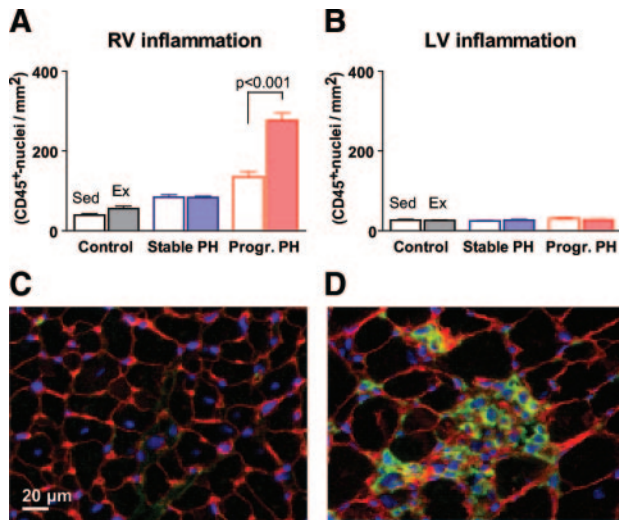
Figure 7. A, Training had a dramatic effect on RV leukocyte infiltration in progressive PH, whereas leukocyte infiltration remained unchanged in stable PH. Moreover, there was a strong significant interaction between training status and PH status ($P<0.001$). B, Inflammation was restricted to the right ventricle only; values for leukocyte infiltration of LV did not differ among the experimental groups and were even lower than RV control values. Bottom, Typical examples of RV leukocyte infiltration (immunofluorescence CD45; $\times100$ magnification) of control-Sed (C) and progressive PH-Ex (D). Green indicates CD45; blue, nuclei; red, cardiomyocyte cell membranes. Notice the clustering of aggregated leukocytes in progressive PH-Ex into the myocardium of the right ventricle. Data are presented as mean±SEM.

moderate RV dilatation that remained stable during the study period. In agreement with this clinical study, we found a marked improvement in exercise endurance in stable PH after training.

In addition, we found that in stable PH the functional improvement after training was associated with enhanced capillarization of the RV. This phenomenon has been described for ischemic heart failure,[20] and 2 recent studies have evaluated the effect of training on cardiac angiogenesis in systemic hypertension.[21,22] In spontaneously hypertensive rats[21] and in rats with angiotensin II–induced hypertension,[22] exercise was found to improve (LV) capillarization by ≈40%. This is somewhat higher than the 25% increase in (RV) capillarization observed in our present study, but the difference may be explained by the lower training intensity and shorter exercise period in our study. A direct link between angiogenesis, hypertrophy, and cardiac function has been shown.[23,24] Insufficient cardiac microvascular growth was recently identified as an important underlying mechanism in the transition from compensatory hypertrophy to heart failure.[23] Moreover, promotion of cardiac angiogenesis was found to normalize the relative capillary deficit, to improve coronary flow reserve, and to restore cardiac dysfunction under chronic pressure overload.[24]

It is likely that the improved endurance in stable PH is also partially attributable to other beneficial effects of exercise that were not studied here. In particular, its effects on skeletal muscle function in PH deserves further exploration in future studies because this effect was shown to be relevant in the rehabilitation of (left) heart failure and chronic obstructive pulmonary disease patients.[25,26]

Worsened Survival After Training Associated With Enhanced RV Inflammation

Traditionally, PH patients were encouraged to limit physical activity, a view that was based mainly on theoretical arguments.[3] Here, we demonstrate that exercise training in progressive PH can indeed be harmful in the case of a poorly adapted RV by augmenting pressure overload–associated RV inflammation.

It is unlikely that RV inflammation is primarily the result of a direct inflammatory effect of monocrotaline on the heart.[27] We found no evidence for LV inflammation, even in rats treated with the highest monocrotaline dose. Furthermore, histology of the RV revealed randomly distributed patches of infiltration rather than a gradual pattern of inflammatory cells diffusing from the (sub)endocardium. For these reasons, RV inflammation in our model is most likely ascribed to chronic RV pressure overload.

To the best of our knowledge, the link between RV inflammation and chronic RV pressure overload has not been investigated yet, neither clinically nor experimentally. However, in patients suffering from acute pulmonary embolism, comparable observations of selective RV inflammation were reported in a postmortem study,[18] and similar findings were observed in experimentally induced acute pulmonary embolism.[28] The mechanistic importance of RV inflammation was demonstrated because suppression of the inflammatory response after acute pulmonary embolism limited RV damage and prevented right heart failure.[29] In these studies, it was suggested that RV inflammation could have been triggered by ischemic injury of the RV or local and/or systemic overproduction of catecholamines.

High RV wall stress might be an alternative explanation, as recently shown in a model of chronic LV pressure overload.[9,30] We observed a similar amount of RV hypertrophy in all PH groups. However, because of larger RV end-diastolic diameter, we found the highest RV wall stress in progressive PH. At resting conditions, RV wall stress was similar in progressive PH-Ex and progressive PH-Sed. Although we could not directly measure RV pressures during exercise, RV afterload probably increased significantly during exercise because of the elevated PVR.[8] Therefore, it is likely that in progressive PH, RV wall stress was higher during exercise. These episodes of elevated wall stress could have triggered RV inflammation because short periods of mechanical stretch (10 minutes) can induce myocardial overexpression of proinflammatory cytokines (like tumor necrosis factor-α), which is followed by leukocyte infiltration.[9,30]

Finally, training might also directly aggravate preexisting inflammation in the myocardium. In viral myocarditis, it is known that exercise augments the inflammatory reaction, enhances cardiac dilatation, and increases its lethality.[31,32]

Our study suggests that RV inflammation in PH may be of pathophysiological importance. Future studies should investigate its relevance for the different causes of clinical pulmonary arterial hypertension.

Limitations

The model of PH that was caused by the use of monocrotaline does not fully replicate the pathophysiology and resulting pulmonary and cardiovascular effects of clinical PH. Therefore, this study should be viewed as a seminal analysis of exercise in stable and progressive PH from which other (clinical) studies should arise. For example, validated clinical determinants that can predict a favorable response to exercise training are currently absent.

The noninvasive estimation of PVR results from several measurements and is therefore susceptible to a large variability. Nevertheless, a close correlation was observed between echocardiography-derived PVR measurements and histological parameters for pulmonary vascular remodeling.

Echocardiography or invasive hemodynamic measurements at the end of the study protocol failed to detect changes that could explain the differences in survival and endurance between the trained and sedentary groups. Echocardiography and invasive hemodynamic measurements, however, were obtained at rest and therefore do not reflect exercise hemodynamics, which probably differed between both groups. Moreover, in progressive PH, hemodynamic measurements at the end of the study protocol were obtained at a stage of terminal right heart failure, which was, however, reached earlier in the trained than in the sedentary group. Hence, the differences in survival between groups are reflected more by the time elapsed to reach right heart failure than the hemodynamic findings at right heart failure.

Conclusions

In our rat model, exercise training was found to be beneficial in stable PH but detrimental in progressive PH. The differential effect is probably due to enhanced RV myocardial capillarization in stable PH and RV myocardial inflammation in progressive PH. Future studies are necessary to address the clinical implications of our findings.

Disclosures

None.

References

1. Chin KM, Rubin LJ. Pulmonary arterial hypertension. *J Am Coll Cardiol.* 2008;51:1527–1538.
2. Badesch DB, Abman SH, Ahearn GS, Barst RJ, McCrory DC, Simonneau G, McLaughlin VV. Medical therapy for pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. *Chest.* 2004;126: 35S–62S.
3. Desai SA, Channick RN. Exercise in patients with pulmonary arterial hypertension. *J Cardiopulm Rehabil Prev.* 2008;28:12–16.
4. Humbert M, Sitbon O, Simonneau G. Treatment of pulmonary arterial hypertension. *N Engl J Med.* 2004;351:1425–1436.
5. Troosters T, Casaburi R, Gosselink R, Decramer M. Pulmonary rehabilitation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2005;172:19–38.
6. Piepoli MF, Davos C, Francis DP, Coats AJ. Exercise training meta-analysis of trials in patients with chronic heart failure (ExTraMATCH). *BMJ.* 2004;328:189–192.
7. Mereles D, Ehlken N, Kreuscher S, Ghofrani S, Hoeper MM, Halank M, Meyer FJ, Karger G, Buss J, Juenger J, Holzapfel N, Opitz C, Winkler J, Herth FF, Wilkens H, Katus HA, Olschewski H, Grunig E. Exercise and respiratory training improve exercise capacity and quality of life in patients with severe chronic pulmonary hypertension. *Circulation.* 2006; 114:1482–1489.
8. Provencher S, Herve P, Sitbon O, Humbert M, Simonneau G, Chemla D. Changes in exercise haemodynamics during treatment in pulmonary arterial hypertension. *Eur Respir J.* 2008;32:393–398.
9. Sun M, Chen M, Dawood F, Zurawska U, Li JY, Parker T, Kassiri Z, Kirshenbaum LA, Arnold M, Khokha R, Liu PP. Tumor necrosis factor-alpha mediates cardiac remodeling and ventricular dysfunction after pressure overload state. *Circulation.* 2007;115:1398–1407.
10. Buermans HP, Redout EM, Schiel AE, Musters RJ, Zuidwijk M, Eijk PP, van Hardeveld C, Kasanmoentalib S, Visser FC, Ylstra B, Simonides WS. Microarray analysis reveals pivotal divergent mRNA expression profiles early in the development of either compensated ventricular hypertrophy or heart failure. *Physiol Genomics.* 2005;21:314–323.
11. Hessel MH, Steendijk P, den Adel B, Schutte CI, van der Laarse A. Characterization of right ventricular function after monocrotaline-induced pulmonary hypertension in the intact rat. *Am J Physiol Heart Circ Physiol.* 2006;291:H2424–H2430.
12. Fenning A, Harrison G, Dwyer D, Rose'meyer R, Brown L. Cardiac adaptation to endurance exercise in rats. *Mol Cell Biochem.* 2003;251: 51–59.
13. Shepherd RE, Gollnick PD. Oxygen uptake of rats at different work intensities. *Pflugers Arch.* 1976;362:219–222.
14. Merklinger SL, Jones PL, Martinez EC, Rabinovitch M. Epidermal growth factor receptor blockade mediates smooth muscle cell apoptosis and improves survival in rats with pulmonary hypertension. *Circulation.* 2005;112:423–431.
15. Handoko ML, Schalij I, Kramer K, Sebkhi A, Postmus PE, van der Laarse WJ, Paulus WJ, Vonk-Noordegraaf A. A refined radio-telemetry technique to monitor right ventricle or pulmonary artery pressures in rats: a useful tool in pulmonary hypertension research. *Pflugers Arch.* 2008; 455:951–959.
16. Henkens IR, Mouchaers KT, Vliegen HW, van der Laarse WJ, Swenne CA, Maan AC, Draisma HH, Schalij I, van der Wall EE, Schalij MJ, Vonk-Noordegraaf A. Early changes in rat hearts with developing pulmonary arterial hypertension can be detected with three-dimensional electrocardiography. *Am J Physiol Heart Circ Physiol.* 2007;293: H1300–H1307.
17. des Tombe AL, van Beek-Harmsen BJ, Lee-de Groot MB, van der Laarse WJ. Calibrated histochemistry applied to oxygen supply and demand in hypertrophied rat myocardium. *Microsc Res Tech.* 2002;58:412–420.
18. Begieneman MP, van de Goot FR, van der Bilt I, Vonk-Noordegraaf A, Spreeuwenberg MD, Paulus WJ, van Hinsbergh V, Visser FC, Niessen HW. Pulmonary embolism causes endomyocarditis in the human heart. *Heart.* 2008;94:450–456.
19. Twisk JWR. *Applied Multilevel Analysis: A Practical Guide.* Cambridge, UK: Cambridge University Press; 2006.
20. Leosco D, Rengo G, Iaccarino G, Golino L, Marchese M, Fortunato F, Zincarelli C, Sanzari E, Ciccarelli M, Galasso G, Altobelli GG, Conti V, Matrone G, Cimini V, Ferrara N, Filippelli A, Koch WJ, Rengo F. Exercise promotes angiogenesis and improves beta-adrenergic receptor signalling in the post-ischaemic failing rat heart. *Cardiovasc Res.* 2008; 78:385–394.
21. Ziada AM, Hassan MO, Tahlilkar KI, Inuwa IM. Long-term exercise training and angiotensin-converting enzyme inhibition differentially enhance myocardial capillarization in the spontaneously hypertensive rat. *J Hypertens.* 2005;23:1233–1240.
22. Belabbas H, Zalvidea S, Casellas D, Moles JP, Galbes O, Mercier J, Jover B. Contrasting effect of exercise and angiotensin II hypertension on in vivo and in vitro cardiac angiogenesis in rats. *Am J Physiol Regul Integr Comp Physiol.* 2008;295:R1512–R1518.
23. Shiojima I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao R, Colucci WS, Walsh K. Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. *J Clin Invest.* 2005; 115:2108–2118.

24. Sano M, Minamino T, Toko H, Miyauchi H, Orimo M, Qin Y, Akazawa H, Tateno K, Kayama Y, Harada M, Shimizu I, Asahara T, Hamada H, Tomita S, Molkentin JD, Zou Y, Komuro I. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. *Nature*. 2007; 446:444–448.

25. Clark AL, Poole-Wilson PA, Coats AJ. Exercise limitation in chronic heart failure: central role of the periphery. *J Am Coll Cardiol*. 1996;28: 1092–1102.

26. Gosselink R, Troosters T, Decramer M. Peripheral muscle weakness contributes to exercise limitation in COPD. *Am J Respir Crit Care Med*. 1996;153:976–980.

27. Akhavein F, St-Michel EJ, Seifert E, Rohlicek CV. Decreased left ventricular function, myocarditis, and coronary arteriolar medial thickening following monocrotaline administration in adult rats. *J Appl Physiol*. 2007;103:287–295.

28. Watts JA, Zagorski J, Gellar MA, Stevinson BG, Kline JA. Cardiac inflammation contributes to right ventricular dysfunction following experimental pulmonary embolism in rats. *J Mol Cell Cardiol*. 2006;41: 296–307.

29. Zagorski J, Gellar MA, Obraztsova M, Kline JA, Watts JA. Inhibition of CINC-1 decreases right ventricular damage caused by experimental pulmonary embolism in rats. *J Immunol*. 2007;179:7820–7826.

30. Kapadia SR, Oral H, Lee J, Nakano M, Taffet GE, Mann DL. Hemodynamic regulation of tumor necrosis factor-alpha gene and protein expression in adult feline myocardium. *Circ Res*. 1997;81:187–195.

31. Gatmaitan BG, Chason JL, Lerner AM. Augmentation of the virulence of murine coxsackie-virus B-3 myocardiopathy by exercise. *J Exp Med*. 1970;131:1121–1136.

32. Ilback NG, Fohlman J, Friman G. Exercise in coxsackie B3 myocarditis: effects on heart lymphocyte subpopulations and the inflammatory reaction. *Am Heart J*. 1989;117:1298–1302.

CLINICAL PERSPECTIVE

The role of exercise training in current clinical management of patients with pulmonary arterial hypertension (PH) remains controversial. Traditionally, these patients were advised to limit their physical activity because exercise was presumed to superimpose an additional right ventricular load on an already compromised heart. Recently, exercise training was found to greatly improve exercise capacity and quality of life in clinically stable PH patients. It remains uncertain, however, if exercise training is beneficial for all PH patients, including the effects on the right ventricle. Therefore, in the present study, we studied the effects of exercise training in a rodent model, inducing 2 distinct clinical phenotypes of PH. Training was found to be beneficial in stable PH with preservation of cardiac function. However, training was detrimental in progressive PH because it accelerated the progression toward right heart failure and induced widespread right ventricular inflammation. These findings imply that disease progression and right ventricular adaptation might be important clinical determinants for favorable response to exercise training in PH. Because clinical PH tends to be a progressive disease, defining "stable" PH might be challenging. In the absence of prospectively validated criteria, PH patients could be classified as stable when there is constancy of clinical findings in repeated evaluations over a 3-month period. These findings should include New York Heart Association classification, 6-minute walk distance, N-terminal prohormone brain natriuretic peptide levels, and right ventricular function/dimensions. Prospective clinical studies should evaluate these criteria for stable PH to translate the findings of the present study and to detect PH patients who will benefit from an exercise training program.