Endothelin receptor blockade combined with phosphodiesterase-5 inhibition increases right ventricular mitochondrial capacity in pulmonary arterial hypertension

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Mouchaers KT, Schalij I, Versteilen AM, Hadi AM, van Nieuw Amerongen GP, van Hinsbergh VW, Postmus PE, van der Laarse WJ, and Vonk-Noordegraaf A. Endothelin receptor blockade combined with phosphodiesterase-5 inhibition increases right ventricular mitochondrial capacity in pulmonary arterial hypertension. Am J Physiol Heart Circ Physiol 297: H200–H207, 2009. First published April 24, 2009; doi:10.1152/ajpheart.00893.2008.—Pulmonary arterial hypertension (PAH) is often treated with endothelin (ET) receptor blockade or phosphodiesterase-5 (PDE5) inhibition. Little is known about the specific effects on right ventricular (RV) function and metabolism. We determined the effects of single and combination treatment with Bosentan [an ET type A (ETa) receptor blocker] and Sildenafil (a PDE5 inhibitor) on RV function and oxidative metabolism in monocrotaline (MCT)-induced PAH. Fourteen days after MCT injection, male Wistar rats were orally treated for 10 days with Bosentan, Sildenafil, or both. RV catheterization and echocardiography showed that MCT clearly induced PAH. This was evidenced by increased RV systolic pressure, reduced cardiac output, increased pulmonary vascular resistance (PVR), and reduced RV fractional shortening. Quantitative histochemistry showed marked RV hypertrophy and fibrosis. Monotreatment with Bosentan or Sildenafil had no effect on RV systolic pressure or cardiac function, but RV fibrosis was reduced and RV capillarization increased. Combination treatment did not reduce RV systolic pressure, but significantly lowered PVR, and normalized cardiac output, RV fractional shortening, and fibrosis. Only combination treatment increased the mitochondrial capacity of the RV, as reflected by increased succinate dehydrogenase and cytochrome c oxidase activities, associated with an activation of PKG, as indicated by increased VASP phosphorylation. Moreover, significant interactions were found between Bosentan and Sildenafil on PVR, cardiac output, RV contractility, PKG activity, and mitochondrial capacity. These data indicate that the combination of Bosentan and Sildenafil may beneficially contribute to RV adaptation in PAH, not only by reducing PVR but also by acting on the mitochondria in the heart.

Bosentan; Sildenafil; mitochondria; oxidative capacity; chronic heart failure

PULMONARY ARTERIAL HYPERTENSION (PAH) is a severe progressive disease of the small lung vasculature leading to increased pulmonary vascular resistance (PVR). The right ventricle (RV) hypertrophies to withstand the rise in PVR and maintain cardiac output (CO) (16, 48). Subsequently, RV contractile dysfunction occurs, and failure may develop (8, 51). Many factors influence the capacity of the RV to adapt to PAH, such as the degree of RV wall stress, myocardial ischemia, or microvascular endothelial dysfunction. However, the exact sequence of events leading the RV toward failure, and the effects of treatments to counteract this process remain uncertain (51).

Various treatments for PAH have been developed over time, which primarily act as vasodilators of the pulmonary vasculature. These treatments include anticoagulant therapy, prostacyclin infusion, endothelin (ET) receptor blockade, and phosphodiesterase-5 (PDE5) inhibition (9, 25). ET receptor blockade by Bosentan and PDE5 inhibition by Sildenafil are both common strategies in PAH treatment (9, 25, 43). The ET system is highly active in PAH and causes sustained vasoconstriction of pulmonary arteries. It increases the mitogenic activity of smooth muscle cells and fibroblasts in the pulmonary vessel wall, thereby decreasing the lumen of pulmonary vessels, also contributing to increased pulmonary vascular resistance (PVR) (24, 40). In addition, it is known that ET receptor expression in the myocardium of the RV increases upon PAH (47). Similarly, PDE5 is overactive in the pulmonary vasculature of PAH patients, where it prevents normal relaxation of smooth muscle cells in the vessel wall by excessive degradation of cGMP. PDE5 inhibition causes relaxation of pulmonary vessels and also exerts antiproliferative effects on pulmonary artery smooth muscle cells (24, 53). In addition, it has been shown that PDE5 is highly expressed in the RV of PAH patients and rats, and that acute inhibition increases RV contractility (35).

Although these reports have shed light on the etiology and treatment of the arterial pathology and pulmonary hypertension, limited information is available regarding counteracting the (mal)adaptation of the heart in PAH (34, 46). Central in this (mal)adaptation is the hypertrophy of RV cardiomyocytes that is needed to generate sufficient power to perfuse the lung vascular bed. However, the increased demand of power output of the RV in PAH requires a rise in O2 delivery to RV cardiomyocytes to maintain sufficient ATP synthesis. The increased O2 consumption and diffusion distances for O2 in hypertrophied cardiomyocytes, in combination with the reduced capillary density, can lead to a mismatch between O2 supply and demand (15, 54). In turn, this can cause hypoxia in individual cardiomyocytes and may lead to cellular damage, contributing to RV failure (45, 51).

Bosentan and Sildenafil have been extensively studied in regard to their effects on pulmonary vessels. However, knowledge about their specific effects on the RV is still limited. We...
hypothesized that treatment with Bosentan, Sildenafil, or their combination affects cardiac function and determinants for RV contractility and oxidative metabolism in a rat reversal model of monocrotaline (MCT)-induced PAH. To this end, we studied RV contractility using echocardiography and measured RV cardiomyocyte hypertrophy as a measure for \(O_2\) diffusion distances. In addition, we studied the RV myoglobin concentration as a parameter for intracellular \(O_2\) diffusion and buffering and RV capillary density as a determinant of \(O_2\) supply capacity. Furthermore, we investigated two mitochondrial enzymes as measures of mitochondrial capacity, changes in cardiomyocyte PKG activity, and the formation of fibrosis in the RV.

MATERIALS AND METHODS

See the online Supplement Material for additional details on reagents and methods.1

Study design. PAH was induced in 28 male Wistar rats by a single injection of MCT (MCT rats; 40 mg/kg sc, Sigma-Aldrich, Zwijndrecht, The Netherlands). After 14 days, PAH rats were randomly assigned to receive oral treatment for 10 days with Bosentan (100 mg·kg\(^{-1}\)·day\(^{-1}\), n = 7; Actelion Pharmaceuticals, Allschwill, Switzerland), Sildenafil (1 mg·kg\(^{-1}\)·day\(^{-1}\), n = 7; Pfizer), or their combination (same dosages, n = 7; Fig. 1). On day 25, rats were euthanized by heart excision under isoflurane anesthesia (>3.5%). All experiments were approved by the Institutional Animal Care and Use Committee and conformed with Helsinki conventions for the use and care of animals.

Echocardiographic and hemodynamic measurements. On days 0, 14, and 25, M-mode echocardiographic images were made of the RV and left ventricle (LV) at midpapillary level and the heart rate (HR) was recorded using an Aloka SSD4000 ultrasonographic system (Biomedic, Almere, The Netherlands; Fig. 1). RV and LV end-systolic diameters (ESDs) and end-diastolic diameters (EDDs) of three cardiac cycles in four different recordings were measured using Scion Image (version 4.0.3.2, Scioncorp). Averaged values of the LVEDD and LVESD were used to calculate stroke volume (SV) and CO by calculating end-systolic and end-diastolic volumes assuming the LV as a prolate spheroid (14, 42). In addition, RV and LV fractional shortening (FS) values were calculated. Before death, the RV systolic pressure (RVSP) was measured using a Millar pressure catheter (Millar) by direct insertion through the RV walls after thoracotomy. Data were obtained using a PowerLab system (AD Instruments, New South Wales, Australia). Systemic blood pressure was monitored using a fluid-filled catheter inserted into the left carotid artery. PVR was estimated according to Poiseuille’s law as previously described (33, 35, 49). Sample densities were expressed as a ratio between phospho-VASP and total VASP, where MCT and MCT + treatment samples are expressed relative to the ratio of the control samples, which was set to 1.

Statistical analysis. Mean values \(\pm SE\) are given. Since comparisons in this study were planned, Student’s \(t\)-test was used to determine differences between means. Two-way ANOVA was used to detect interactions between Bosentan and Sildenafil treatments. \(P\) values of \(<0.05\) were considered to be statistically significant.

RESULTS

Effects of treatment on hemodynamics, body mass, and lung mass. Table 1 shows hemodynamic parameters and body and lung masses of the various groups of animals studied. MCT clearly induced PAH, which was clearly demonstrated by the higher RVSP on day 25 than in control animals. Neither single treatment with either Bosentan or Sildenafil nor simultaneous treatment with both drugs significantly reduced MCT-induced RVSP significantly. Nontreated MCT rats and both groups of single drug-treated MCT rats had lower systemic blood pressures than control animals.

Body masses of all PAH rats, including treated rats, were significantly lower on day 25 compared with their control counterparts (Table 1). In addition, lung wet masses were significantly increased in MCT-, Bosentan + MCT-, and Sildenafil + MCT-treated rats, whereas the combination treatment normalized lung mass.

Effects of treatment on RV and LV dimensions and contractility, CO, SV, and PVR. To evaluate the effects of MCT-induced PAH and treatment by Bosentan and Sildenafil on the heart, functional echocardiography was performed in anesthetized rats. Representative pictures of M-mode echocardiographic recordings of the RV are shown in Fig. 1. Echocardiographic data are shown in Table 1. After 25 days, MCT rats showed marked dilation of the RV, as demonstrated by increased RVEDDs and RVESDs. RV FS significantly decreased. RVEDD and RVESD values of Bosentan + MCT- and Sildenafil + MCT-treated rats were not different from those of untreated MCT rats, but the calculation of RV FS revealed a further decrease. Combined Bosentan and Sildenafil treatment of MCT rats resulted in less RV dilation, as evidenced by smaller RVEDD and RVESD values, resulting in normalized RV FS. LVEDD and LVESD significantly decreased upon MCT exposure and further decreased upon Bosentan monotherapy, which was not observed in Silde-

\(^1\) Supplemental Material for this article is available online at the American Journal of Physiology-Heart and Circulatory Physiology website.
Menadione and Sildenafil treatment, whereas the combination treatment significantly lowered PVR. On days 0 and 14, no differences in RV systolic pressure, mmHg; 28±2* vs. control; ‡P<0.005 vs. control; †P<0.05‡ vs. 2,687*‡ 0.0005 vs. control; NS vs. 10* NS vs. 7N S vs. control. Right heart catheterization was performed to measure RV systolic pressure and LV end-diastolic diameter, respectively; RV EDD, RV end-diastolic diameter; RVESD, RV end-systolic diameter; FS, fractional shortening; Vertical bars show the RV end-diastolic diameter (EDD) and end-systolic diameter (ESD). MCT rats showed RV dilation, as indicated by the absence of cytochrome c release in cardiomyocytes (data not shown). Typical examples of sections of myocardial tissue stained for hematoxylin and eosin, myoglobin, collagen type IV, Picrosirius red, SDH, and cytochrome c oxidase are shown in Fig. 2, whereas Fig. 3 shows the quantification of these data for the various groups of animals. MCT clearly induced RV hypertrophy, indicated by an increase in the value of the interaction between Bosentan and Sildenafil. P Values are means ± SE; n = 5–7 rats/group (also see the online Supplemental Material). RV, right ventricle; LV, left ventricle; MCT, monocrotaline; RV EDD, RV end-diastolic diameter; RVESD, RV end-systolic diameter; CO, cardiac output; SV, stroke volume; FS, fractional shortening; NS, not significant. *P < 0.05 and ‡P < 0.005 vs. control; †P < 0.05 and $P < 0.005 vs. untreated MCT rats. Values are means ± SE; n = 5–7 rats/group (also see the online Supplemental Material). RV, right ventricle; LV, left ventricle; MCT, monocrotaline; RV EDD, RV end-diastolic diameter; RVESD, RV end-systolic diameter; CO, cardiac output; SV, stroke volume; FS, fractional shortening; NS, not significant. *P < 0.05 and ‡P < 0.005 vs. control; †P < 0.05 and $P < 0.005 vs. untreated MCT rats.

Table 1. Hemodynamic and echocardiographic data for the RV and LV, body mass, and lung mass of rats from the control, untreated MCT, MCT + Bosentan, MCT + Sildenafil, and combination treatment groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RV Systolic Pressure, mmHg</th>
<th>Systemic Pressure, mmHg</th>
<th>RV EDD, mm</th>
<th>RVESD, mm</th>
<th>RV FS, %</th>
<th>LVEDD, mm</th>
<th>LVESD, mm</th>
<th>LV FS, %</th>
<th>Stroke Volume, µL</th>
<th>Cardiac Output, ml/min</th>
<th>Pulmonary Vascular Resistance, dyne·s·cm⁻²</th>
<th>Heart Rate, beats/min</th>
<th>Body Mass, g</th>
<th>Lung Mass, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28±2*</td>
<td>4.18±0.52*</td>
<td>1.07±0.06*</td>
<td>5.7±3*</td>
<td>6.75±0.17*</td>
<td>2.97±0.17*</td>
<td>56±2</td>
<td>296±21</td>
<td>116±10*</td>
<td>15,005±780*</td>
<td>392±13*</td>
<td>363±98</td>
<td>1.69±0.11*</td>
<td>2.41±0.47*</td>
</tr>
<tr>
<td>MCT (Placebo)</td>
<td>62±7*</td>
<td>4.18±0.52*</td>
<td>2.39±0.38*</td>
<td>44±3*</td>
<td>5.86±0.22*</td>
<td>3.20±0.20*</td>
<td>61±2</td>
<td>201±21</td>
<td>201±21</td>
<td>14,224±8,451*</td>
<td>345±15*</td>
<td>311±12*</td>
<td>2.06±0.06*</td>
<td>2.21±0.11*</td>
</tr>
<tr>
<td>MCT + Bosentan</td>
<td>64±5*</td>
<td>5.24±0.50*</td>
<td>3.52±0.38*</td>
<td>33±3*</td>
<td>5.43±0.21*</td>
<td>1.86±0.15*</td>
<td>66±2</td>
<td>164±18</td>
<td>164±18</td>
<td>69,276±8,913*</td>
<td>291±11*</td>
<td>311±12*</td>
<td>2.11±0.06*</td>
<td>2.21±0.11*</td>
</tr>
<tr>
<td>MCT + Sildenafil</td>
<td>55±5*</td>
<td>4.91±0.44*</td>
<td>3.16±0.27*</td>
<td>35±2*</td>
<td>5.99±0.45</td>
<td>2.39±0.26</td>
<td>60±4</td>
<td>228±44</td>
<td>228±44</td>
<td>60,350±5,983*</td>
<td>343±16*</td>
<td>305±12*</td>
<td>3.05±0.12*</td>
<td>3.12±0.13*</td>
</tr>
<tr>
<td>MCT + Bosentan + Sildenafil</td>
<td>55±10*</td>
<td>&lt;0.0001</td>
<td>3.16±0.27*</td>
<td>35±2*</td>
<td>6.65±0.18*</td>
<td>2.39±0.26</td>
<td>61±3</td>
<td>234±19</td>
<td>234±19</td>
<td>61±3</td>
<td>234±19</td>
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**Fig. 1.** Top: study design. Pulmonary arterial hypertension (PAH) was induced in male Wistar rats by an injection of monocrotaline (MCT; 40 mg/kg sc). Fourteen days after MCT treatment, rats were orally treated with Bosentan (Bos), Sildenafil (Sil), or both drugs for 10 days. On days 0, 14, and 25, echocardiography was performed to monitor right ventricular (RV) and left ventricular (LV) function. On day 25, right heart catheterization was performed to measure RV systolic pressure (RVSP), and, subsequently, hearts were excised for quantitative histochemistry. Bottom: representative echocardiographic images of the RV obtained in the M-mode, which used to calculate RV fractional shortening. Vertical bars show the RV end-diastolic diameter (EDD) and end-systolic diameter (ESD).
mean RV cardiomyocyte CSA. Bosentan, Sildenafil, and also the combination treatment further increased cardiomyocyte hypertrophy (Figs. 2A and 3A). The myoglobin concentration did not differ between cardiomyocytes of control, nontreated, and drug-treated MCT rats (Figs. 2B and 3B). Nontreated MCT rats showed a trend toward an increase in capillary density. The number of capillaries per cardiomyocyte increased significantly in MCT rats exposed to both single drug and combination treatments compared with control rats (Figs. 2C and 3C). In addition, MCT increased the formation of fibrosis in the RV, which reduced significantly upon single treatments with either Bosentan or Sildenafil and normalized upon combination treatment (Figs. 2D and 3D). The mean RV cardiomyocyte SDH activity of control, nontreated MCT, or single drug-treated MCT rats was similar, whereas the combination treatment significantly increased SDH activity (Figs. 2E and 3E). Cytochrome c oxidase activity revealed a similar pattern (Figs. 2F and 3F). SDH and cytochrome c oxidase activities were proportional ($P < 0.0001$, $r^2 = 0.66$).

For comparison, mean LV cardiomyocyte CSA, myoglobin concentrations, and capillary densities were similar in all groups (382 ± 13 μm², 0.74 ± 0.03 mM, and 1.60 ± 0.03 capillaries/cell, respectively, $n = 31$). However, LV fibrosis significantly increased in nontreated MCT rats compared with control rats (2.9 ± 0.8% vs. 1.1 ± 0.4%, respectively, $P = 0.0002$) and was only lowered by the combined treatment of Bosentan and Sildenafil (to 1.9 ± 0.3%, $P = 0.012$ vs. MCT rats). In addition, SDH activity increased in Sildenafil- and combination-treated rats [absorbance (A) at 660 nm = 0.172 ± 0.006, $n = 14$; pooled value for the two groups compared with...
pooled control, nontreated, and Bosentan treated MCT rats, which were similar: \( A_{660\text{nm}} = 0.133 \pm 0.005, P < 0.0001, n = 17 \), which was also observed for cytochrome c oxidase activity \( A_{436\text{nm}} = 0.223 \pm 0.004, n = 14 \); pooled value for the two groups compared with pooled control, nontreated, and Bosentan treated MCT rats, which were similar: \( A_{436\text{nm}} = 0.193 \pm 0.005, P < 0.0001, n = 17 \).

Effect of treatment on RV PKG activity. Subsequently, we studied whether the effects of treatment on the RV could be explained by a change in cGMP. To this end, the activity of PKG in the RV was measured by determining the degree of Ser239 phosphorylation of VASP. Western blot analysis showed that the RV of MCT rats revealed no difference in RV PKG activity compared with control rats. This shows that PAH induction by MCT itself does not alter PKG activity in RV cardiomyocytes. However, while single treatment with Bosentan or Sildenafil had no significant effect on VASP phosphorylation, the combination of both drugs significantly increased PKG activity by 4.6-fold (Fig. 4).

Interactions between Bosentan and Sildenafil. Two-way ANOVA detected significant interactions between Bosentan and Sildenafil treatment. In addition to those shown Table 1, interactions were found for mean RV CSA \( (P = 0.034) \), Ser239 phosphorylation of VASP \( (P = 0.023) \), and cytochrome c oxidase activity \( (P = 0.0035) \).

DISCUSSION

The main finding of our study is that, in this model of MCT-induced PAH, the combination of orally administered Bosentan and Sildenafil, but not single treatments, improved the RV adaptation to severe PAH with respect to the number of
capillaries per cardiomyocyte and mitochondrial capacity, as reflected by SDH and cytochrome c oxidase activities. This was associated with a lowering of PVR and normalization of CO, RV FS, and fibrosis.

In this PAH model, RVSP is already elevated by day 14 (22). We specifically chose to study the treatment of already developed PAH and examined the effects of 10 days of treatment with Bosentan and Sildenafil. Compared with previous studies (11, 23, 46), we used a relatively short period of treatment and low dosages of drugs, which may have precluded effects on the reduction of RVSP. In addition, compared with these studies, we used relatively young rats, which may have resulted in more progressive PAH. Furthermore, the biological effects for Sildenafil may differ depending on the experimental model for pulmonary trunk banding was not able to attenuate PAH development; in fact, it increased RV hypertrophy. In contrast, RV SDH and cytochrome c oxidase activities increased in proportion to the hypertrophy induced by MCT. In contrast, RV SDH and cytochrome c oxidase activities increased only in rats treated simultaneously with both Bosentan and Sildenafil. As the only combination treatments with Bosentan and Sildenafil resulted in a major improvement of RV function, we conclude that increased cardiomyocyte power output is an additional mechanism contributing to restored RV function in PAH rats. It is of interest to note that the mitochondrial capacity in the RV was not altered by the administration of MCT itself. This indicates that the restoration of RV function reflects a compensatory reaction rather than inhibiting the cause of PAH.

Our findings also indicate synergistic actions of Bosentan and Sildenafil on several parameters, such as PVR, RV dimensions and contractility, and cardiomyocyte size and oxidative capacity to synthesize ATP. These parameters of maximal O2 demand are reflected by CSA, SDH activity, or cytochrome c oxidase activity. The activities of SDH and cytochrome c oxidase are proportional, and we (15) have previously shown that SDH activity is proportional to the maximum rate of O2 consumption. We found that all treatments increased CSA further compared with CSA, SDH activity, or cytochrome c oxidase activity. The activities of SDH and cytochrome c oxidase are proportional, and we (15) have previously shown that SDH activity is proportional to the maximum rate of O2 consumption. We found that all treatments increased CSA further compared with the hypertrophy induced by MCT. In contrast, RV SDH and cytochrome c oxidase activities increased only in rats treated simultaneously with both Bosentan and Sildenafil. As the only combination of Bosentan and Sildenafil resulted in a major improvement of RV function, we conclude that increased cardiomyocyte power output is an additional mechanism contributing to restored RV function in PAH rats. It is of interest to note that the mitochondrial capacity in the RV was not altered by the administration of MCT itself. This indicates that the restoration of RV function reflects a compensatory reaction rather than inhibiting the cause of PAH.

Our findings also indicate synergistic actions of Bosentan and Sildenafil on several parameters, such as PVR, RV dimensions and contractility (FS), RV hypertrophy, cytochrome c oxidase activity, and CO. Because Bosentan and Sildenafil affect different pathways involved in PAH pathophysiology, this suggests that the synergy of their intracellular signals is particularly important in counteracting the adverse effects of PAH on RV function. Previous clinical observations have shown normalization of NO bioavailability by Bosentan in PAH (20). It is likely that PDE5 inhibition by Sildenafil augments the NO-mediated effects. The elevated PDE5 in the
RV of PAH patients and MCT rats (35) would locally reduce cGMP generated by NO-stimulated guanylate cyclase. Such synergy of Bosentan and Sildenafil on NO/cGMP-mediated processes may also underlie the observed improvement of mitochondrial density reflected by SDH and cytochrome c oxidase activities. Indeed, it has been shown that NO stimulates mitochondrial biogenesis through the activation of the mitochondrial transcription factors peroxisome proliferator-activated receptor-γ coactivator-1α, nuclear respiratory factor-1, and mitochondrial transcription factor A in a cGMP-dependent manner (10, 36, 37). We therefore investigated whether the combination of Bosentan and Sildenafil would be able to augment the NO-cGMP pathway by investigating the RV Ser239 phosphorylation of VASP. Indeed, we found an increase in RV VASP phosphorylation only with the combination of Bosentan and Sildenafil and not with the single treatments. These data confirm the synergism and indicate that an increase in mitochondrial capacity is likely an effect of the activation of PKG through the rescue of the NO-cGMP pathway.

The observed effects in our study were predominant for the RV, as treatments did not affect LV histological characteristics except for SDH and cytochrome c oxidase activities in both groups that received Sildenafil. Recent studies have shown that Sildenafil is able to reduce the production of ROS by mitochondria (17) and that it exerts cardioprotective effects through opening of Ca2⁺-sensitive, ATP-sensitive K⁺ channels (13, 52). Whether this is related to an increase in oxidative capacity remains to be investigated. Fibrosis was also reduced upon single and combination treatments in both ventricles. Although it is generally believed that MCT only affects the RV, evidence is increasing indicating that the LV is also affected by MCT through the activation of various neurohumoral factors (1, 31). In addition, we observed altered LV dimensions as indicated by reduced LVEDD and LVESD, which was also observed in Bosentan-treated rats. This suggests that the LV of PAH rats was also subjected to mechanical changes secondary to the pressure-overloaded RV, such as impaired LV filling (30, 32).

In conclusion, we demonstrated that the combination of ET receptor blockade by Bosentan and PDE5 inhibition by Sildenafil, at dosages that are ineffective in single treatments, is able to increase RV mitochondrial enzyme activity in a progressive model of MCT-induced PAH, likely through the activation of PKG by enforcement of the NO-cGMP pathway. This was associated with a lowering of PVR, a restoration of RV contractility, a normalization of RV fibrosis, and a significant increase in RV capillarization, resulting in the maintenance of normal CO. Our data confirm the synergism between Bosentan and Sildenafil and indicate that combination treatment with both drugs may act at the mitochondrial level in the heart.

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