Selective Class I HDAC Inhibition Suppresses Hypoxia-Induced Cardiopulmonary Remodeling Through an Anti-Proliferative Mechanism

Maria A. Cavasin, Kim Demos-Davies, Todd R. Horn, Lori A. Walker, Douglas D. Lemon, Nicholas Birdsey, Mary C. M. Weiser-Evans, Jules Harral, David C. Irwin, Adil Anwar, Michael E. Yeager, Min Li, Peter A. Watson, Raphael A. Nemenoff, Peter M. Buttrick, Kurt R. Stenmark, and Timothy A. McKinsey

Department of Medicine, Division of Cardiology, University of Colorado Denver, Aurora, CO
Department of Medicine, Division of Endocrinology, Metabolism and Diabetes, University of Colorado Denver, Aurora, CO
Department of Medicine, Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO
Department of Pediatrics, Division of Pulmonary and Critical Care Medicine, University of Colorado Denver, Aurora, CO
Denver VA Medical Center, Denver, CO

Abstract

Rationale—Histone deacetylase (HDAC) inhibitors are efficacious in models of hypertension-induced left ventricular (LV) heart failure. The consequences of HDAC inhibition in the context of pulmonary hypertension (PH) with associated right ventricular (RV) cardiac remodeling are poorly understood.

Objective—This study was performed to assess the utility of selective small molecule inhibitors of class I HDACs in a pre-clinical model of PH.

Methods and Results—Rats were exposed to hypobaric hypoxia for 3 weeks in the absence or presence of a benzamide HDAC inhibitor, MGCD0103, which selectively inhibits class I HDACs –1, –2 and –3. The compound reduced pulmonary arterial pressure (PAP) more dramatically than tadalafil, a standard-of-care therapy for human PH that functions as a vasodilator. MGCD0103 improved pulmonary artery (PA) acceleration time (PAAT) and reduced systolic notching of the PA flow envelope, suggesting a positive impact of the HDAC inhibitor on pulmonary vascular remodeling and stiffening. Similar results were obtained with an independent class I HDAC-selective inhibitor, MS-275. Reduced PAP in MGCD0103-treated animals was associated with blunted pulmonary arterial wall thickening due to suppression of smooth muscle cell proliferation. RV function was maintained in MGCD0103 treated animals. Although the class I HDAC inhibitor only modestly reduced RV hypertrophy, it had multiple beneficial effects on the RV, which

Address correspondence to TAM: T. A. McKinsey, Tel: 303-724-5476, Fax: 303-724-5450, timothy.mckinsey@ucdenver.edu.

Publisher’s Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosures
No conflicts of interest exist for the authors.
included suppression of pathological gene expression, inhibition of pro-apoptotic caspase activity, and repression of pro-inflammatory protein expression.

**Conclusions**—By targeting distinct pathogenic mechanisms, isoform-selective HDAC inhibitors have potential as novel therapeutics for PH that will complement vasodilator standards-of-care.

**Keywords**
Histone deacetylase; pulmonary hypertension; proliferation

**Introduction**

In patients with pulmonary hypertension (PH), restricted blood flow through the pulmonary arterial circulation due to increased pulmonary vascular resistance often results in right heart failure. Despite recent advances in the treatment of PH, the 5-year mortality rate for individuals with this disease still approaches 50%, highlighting an urgent need for novel therapeutics. Current standards-of-care (SOC) for patients with PH typically involve the use of vasoactive drugs, including endothelin receptor antagonists (ERAs), phosphodiesterase-5 (PDE-5) inhibitors, and prostacyclins. More effective therapeutic strategies will likely be based on the combined use of vasodilators and agents that target distinct pathogenic mechanisms in PH, such as pulmonary vascular inflammation and fibrosis as well as aberrant proliferation of smooth muscle cells, endothelial cells and fibroblasts in the lung vasculature. Additionally, since RV failure is the cause of death in the majority of PH patients, and it is unclear whether SOC for LV dysfunction (e.g., beta-blockers and angiotensin converting enzyme inhibitors) are effective for RV failure, increased emphasis needs to be placed on elucidating pathogenic mechanisms in this chamber of the heart. Given the multitude of cell types and signaling cascades governing pulmonary vascular and associated right ventricular (RV) remodeling, it is predicted that effective therapeutic strategies for PH will involve targeting distal signaling mediators. Histone deacetylases (HDACs) may represent such targets.

Histone deacetylases (HDACs) control cell proliferation, inflammation and fibrosis by catalyzing removal of acetyl groups from lysine residues in a variety of proteins. The 18 mammalian HDACs are encoded by distinct genes and are grouped into four classes. Two broad-spectrum HDAC inhibitors are approved for the treatment of cancer. One of these compounds, SAHA, as well as other ‘pan’-HDAC inhibitors have been shown to be efficacious in animal models of left ventricular (LV) dysfunction, suggesting unforeseen potential for HDAC inhibitors for the treatment of heart failure. However, a recent report of findings in a model of pulmonary artery banding (PAB) suggested that pan-HDAC inhibitors have deleterious effects on the RV, casting doubt on the utility of this class of compounds for patients with right-sided heart failure due to diseases such as PH.

Here, we tested the hypothesis that selective inhibition of a subset of HDACs will provide a safe and effective means of treating PH and RV remodeling. MGC0103, a small molecule HDAC inhibitor that is in clinical development for cancer and is highly selective for class I HDACs −1, −2 and −3, was tested for efficacy in a rat model of PH induced by chronic hypoxia. Rationale for using this compound was based on prior studies implicating class I HDACs in the control of LV remodeling, and because of the well-described role for this HDAC class in the control of aberrant cell proliferation, such as is found in lung vasculature of patients with PH. The data demonstrate that class I HDAC inhibition significantly reduces pulmonary arterial pressure (PAP) through a mechanism involving suppression of pulmonary vascular smooth muscle cell proliferation, while maintaining RV function and blocking cellular and molecular processes that contribute to RV failure.
Another class I HDAC inhibitor that is in clinical development, MS-275, also effectively reduced hypoxia-induced PH. These results highlight the potential of isoform-selective HDAC inhibitors for the treatment of cardiovascular diseases.

Methods

An expanded Methods section is available in the Online Data Supplement

Experimental animals

All animal experiments were conducted in accordance with the National Institutes Health ‘Guide for the Care and Use of Laboratory Animals’, and were approved by the Institutional Animal Care and Use Committee at the University of Colorado Denver. Ten week-old male Sprague Dawley (SD) rats weighing 250–280g (Charles River Laboratories) were used for all studies. MGCD0103 and MS-275 (Selleck) were delivered every other day via daily intraperitoneal (i.p.) injection at concentrations of 10 mg/kg and 3 mg/kg, respectively, in a 50:50 DMSO:PEG-300 vehicle. Tadalafil (Sequoia Research Products, 10 mg/kg) was dosed daily by oral gavage in a 20% Cremophor/H₂O vehicle (dose volume 2 ml/kg). MGCD0103, MS-275 and tadalafil administration began the day animals were placed in chambers. Normoxic and hypoxic control animals were dosed on the same schedule with compound vehicles. Animals were sacrificed 20 hrs post-compound dosing, unless otherwise indicated.

Hemodynamic analysis

Echocardiographic analyses were performed using a Vevo770 (VisualSonics). Pulse-wave Doppler of pulmonary outflow was recorded in the parasternal short-axis view at the level of the aortic valve. Baseline measurements were obtained one day prior to placing animals in chambers. PAP was measured with a Millar catheter placed in the main pulmonary artery via the RV; correct placement of the catheter was confirmed by observing a significant rise in diastolic pressure as the catheter moved out of the ventricle. Systemic blood pressure was monitored with another pressure catheter inserted in the femoral artery. Cardiac performance was assessed using a pressure-volume system (Scisense). For analyses, animals were anesthetized using 2% isoflurane and their body temperature was maintained at 37°C. Hemodynamic analyses were performed ~20 – 24 hrs following the final dose of compound (MGCD0103 and MS-275) or 2 hrs post-dosing (tadalafil). Total pulmonary vascular resistance index (PVRI) was calculated as mPAP/cardiac index (CI), where CI = cardiac output/body weight, as described previously. For data from all in vivo studies, GraphPad Prism software was used to generate graphs and analyze data. ANOVA with Bonferroni’s post-test (p<0.05) was used to determine statistical differences between groups. Rats presented no health concerns associated with compound treatment. Animals were monitored daily and showed no evidence of paleness in eyes, nose or skin, which are the most common signs of hematological toxicities. Rats were alert and conducted normal activities such as eating, drinking and grooming.

Results

To assess the role of class I HDACs in pulmonary hypertension and RV remodeling, adult SD rats were housed in a hypobaric chamber to simulate an altitude of 18,000 feet above sea level and create a hypoxic environment (10% O₂). Normoxic control rats were maintained in chambers simulating sea level (21% O₂). Animals were treated with compound vehicle or MGCD0103, a selective inhibitor of class I HDACs that is currently in clinical trials for the treatment of cancer (Figure 1A). Normoxic and hypoxic control rats were dosed with compound vehicle alone. Rats receiving MGCD0103 gained weight at a rate similar to vehicle controls, indicating that the compound was well tolerated (Figure 1B). Enzymatic
assays with lung and RV homogenates confirmed that MGCD0103 selectively inhibited class I but not class IIa HDACs (Figure 1C and 1D). MGCD0103 did not increase tubulin acetylation in lung (Figure 1E) or heart (not shown), indicating that the compound did not inhibit the tubulin deacetylase, HDAC6.

Three weeks of hypoxia resulted in a ~2-fold increase in PA systolic pressure (PASP), as determined by placement of a Millar catheter into the pulmonary artery (Figure 2A). MGCD0103 significantly reduced PASP as well as PA pulse pressure (PAPP), suggesting that the compound increased arterial compliance in the lungs of hypoxic rats (Figures 2A and B). Mean PAP (mPAP) values correlated with PASP and PAPP (Figure 2C). Systemic blood pressure was unaffected by MGCD0103 (Online Table II), and MGCD0103 had no impact on pulmonary pressures in normoxic rats (Online Figures IA – 1C). The class I HDAC inhibitor reduced PAP more effectively than tadalafil, a PDE5 inhibitor used to treat patients with PH (Figure 2D).

A follow-up study assessed effects of MGCD0103 on RV function and pulmonary blood flow in hypoxic rats. Importantly, cardiac output (CO) was maintained in animals receiving the class I HDAC inhibitor (Figure 3A), ruling out the possibility that the observed reduction in PAP was a consequence of compound-mediated impairment of cardiac function. Consistent with the findings shown in Figure 2, MGCD0103 also significantly reduced pulmonary vascular resistance in hypoxic rats (Figure 3B). Pressure-volume analyses confirmed that MGCD0103 did not negatively impact RV function (Table 1). In line with the PAP measurements, Doppler echocardiography revealed reduced pulmonary artery acceleration time (PAAT) and velocity time integral (VTI) in hypoxic rats, which was rescued by MGCD0103 (Figures 3C and 3D). Reduced pulmonary vascular compliance often causes transient cessation of forward PA blood flow during systole, which is detected by Doppler as a “notch” in the signal. Systolic notching was readily detected in hypoxic control rats, but not in animals treated with MGCD0103 (Figure 3E).

Biochemical, morphological and histological analyses were performed to further address the action of MGCD0103 in the RV. Consistent with a role for class I HDACs in RV remodeling, HDAC1, 2 and 3 protein levels were elevated in RVs from hypoxic rats and normalized by MGCD0103 treatment (Figures 4A and 4B). Surprisingly, class I HDAC inhibition only modestly reduced RV hypertrophy (Figure 4C and Online Table III). However, RV expression of genes associated with pathological hypertrophy, including brain natriuretic peptide (BNP) and α-skeletal actin, was reduced by MGCD0103 (Figure 4D). Tadalafil also reduced RV hypertrophy (Supplemental Figure 2A – C). In normoxic rats, MGCD0103 did not affect expression of class I HDACs in the RV and did not reduce RV mass or expression of BNP or α-skeletal (Supplemental Figure 3A – D).

In contrast to the increase in RV cell death observed in animals treated with a pan-HDAC inhibitor, selective inhibition of class I HDACs with MGCD0103 blocked induction of pro-apoptotic caspase activity in RVs of hypoxic rats (Figure 4E and 4F). Given the role of inflammation in RV remodeling, and the anti-inflammatory actions of HDAC inhibitors, a cytokine/chemokine protein array was performed with RV homogenates from rats treated with vehicle or MGCD0103. MGCD0103 blunted hypoxia-mediated induction of interleukin-1β, interleukin-2 (IL-2) and cytokine-Induced neutrophil chemoattractant-2 (CINC-2) protein levels in the RV (Figure 4G), suggesting that class I HDAC inhibition blocked activation/recruitment of macrophages, T lymphocytes and neutrophils. Results from the entire array are shown in the data supplement (Supplemental Figure 4).

As shown in Figure 5, an independent class I HDAC inhibitor, MS-275, also effectively blunted hypoxia-induced pulmonary hypertension. MS-275 also reduced hypoxia-mediated
RV hypertrophy (Figure 5C and Online Table III). Like MGCD0103, MS-275 has a benzamide warhead that confers selectivity for class I HDACs. However, linker and surface recognition domains in MS-275 are distinct from those of MGCD0103. Given that these compounds have distinct chemical structures, the results suggest that the reduction in PAP mediated by MGCD0103 and MS-275 is due to selective inhibition of class I HDACs rather than an off-target effect.

To begin to address the mechanism by which class I HDAC inhibition reduces PAP in the setting of hypoxia, experiments were performed to determine whether MGCD0103 exerts acute vasodilator effects. Pulmonary artery strips (1.5 mm X 200 µm) were isolated from adult SD rats, attached to a force transducer, and stimulated to contract with potassium. Acute treatment with MGCD0103 failed to augment potassium-mediated contraction of pulmonary arteries under these conditions, whereas sodium nitroprusside (SNP, 1 µM), which directly activates guanylate cyclase, effectively relaxed the contraction (Figure 6A). Consistent with these findings, acute administration of MGCD0103 (2 or 20 hrs) in treatment-naïve, hypoxic rats did not lower PAP (Figure 6B).

Medial thickening due to abnormal proliferation of PASMCs contributes to the pathogenesis of PH. Given the essential role for class I HDACs in the control of cell proliferation, analyses were performed to determine whether efficacy of MGCD0103 in the PH model was linked to reduced pulmonary vascular remodeling. Hypoxia-mediated medial thickening of vessels in the lung was inhibited by MGCD0103 (Figure 7A, upper panel), which was associated with reduced numbers of proliferating cells (presumably SMCs) in this vessel compartment (Figure 7A, lower panel). Quantification of pulmonary arterial medial thickness revealed complete normalization in MGCD0103 treated rats (Figure 7C); MGCD0103 had no effect on medial thickness of pulmonary vessels from normoxic rats (Supplemental Figure 5A). In contrast to MGCD0103, tadalafil failed to significantly reduce hypoxia-dependent medial thickening of pulmonary arterials (Supplemental Figure 5B).

Studies with MGCD0103 and cultured cells confirmed that the class I HDAC inhibitor directly suppresses PASMC proliferation through a mechanism involving induction of genes that promote cell cycle arrest, including FoxO3a and p27 (Figure 7C – E). MGCD0103 also reduced FoxO3a phosphorylation at threonine 32 in PASMCs cultured under hypoxic conditions (Figure 7D, lane 4); phosphorylation of this site promotes nuclear export of FoxO3a, resulting in activation of genes that promote cell proliferation. Taken together, the data suggest that class I HDAC inhibition improves pulmonary hemodynamics through an anti-proliferative mechanism.

Discussion

Here, we demonstrate in vivo efficacy of selective class I HDAC inhibitors in a pre-clinical model of PH. Two class I HDAC inhibitors, MGCD0103 and MS-275, reduced hypoxia-mediated PH in rats in a manner that correlated with suppression of medial thickening of pulmonary arteries and inhibition of SMC proliferation in these vessels. Reduced PASMC proliferation upon class I HDAC inhibition was due, in part, to upregulation of the anti-proliferative transcription factor, FoxO3a. Importantly, we also demonstrated that RV function was maintained in the face of class I HDAC inhibition, and that indices of adverse ventricular remodeling (e.g., myocyte apoptosis and inflammation) were blunted by selective inhibition of class I HDACs. This is in contrast to what was previously observed with the pan-HDAC inhibitor, trichostatin A (TSA), and supports the hypothesis that isoform-selective HDAC inhibition will be safer than general HDAC inhibition in the setting of RV pressure overload. Both MGCD0103 (Mocetinostat) and MS-275 (Entinostat) are in
clinical development for cancer and are well tolerated by humans, thus highlighting the translational potential of the present findings.

PH is associated with dramatic structural remodeling of the pulmonary vasculature. Remodeling of pulmonary arteries is due, in part, to abnormal proliferation of PASMCs, resulting in muscularization and stenosis of the vessels and thereby increasing pulmonary vascular resistance. The remodeling process is exacerbated by aberrant proliferation of other cell types, including endothelial cells and fibroblasts, as well as vascular inflammation and adventitial fibrosis. As such, it has been proposed that anti-proliferative agents should be used in combination with vasodilators for the treatment of PH, and recent clinical trials with anti-cancer agents such as the tyrosine kinase inhibitor imatinib are addressing this hypothesis.

HDAC inhibitors are in use for the treatment of cancer based on their ability to block proliferation and stimulate apoptosis of transformed cells. Class I HDACs (−1, −2 and −3), which reside in the nucleus and regulate epigenetic processes through deacetylation of nucleosomal histones, appear to be the HDAC isoforms that are primarily responsible for cell cycle control. In addition to their anti-cancer actions, clinical and preclinical studies have revealed that HDAC inhibitors potentiate inflammation, fibrosis and restenosis, and have beneficial effects on the LV in the setting of increased afterload. Furthermore, we found class I HDAC inhibition blocks the persistent, pro-inflammatory phenotype of pulmonary adventitial fibroblasts derived from hypoxic calves. Given these findings, HDAC inhibitors appear to be ideally suited for the treatment of PH, and the results with MGCD0103 and MS-275 described here support this notion.

Two reports have addressed effects of HDAC inhibitors in models of RV remodeling. Valproic acid was shown to block RV cardiac hypertrophy in response to pulmonary artery banding (PAB), as well as in the setting of pulmonary hypertension caused by monocrotaline-induced lung injury. In contrast, TSA failed to block hypertrophy in response to PAB, and actually appeared to worsen RV function. Valproic acid is a weak HDAC inhibitor that has many additional pharmacological activities, including regulation of ion channels, glycogen synthase kinase-3β and mitogen activated protein kinases. Thus it is unclear whether the beneficial effects of this compound on the RV were a direct consequence of HDAC inhibition. TSA is a potent, pan-HDAC inhibitor. The deleterious effects of this compound on the RV (e.g., decreased cardiac output, increased RV dilatation and apoptosis) could be a reflection of a protective role for an HDAC(s) in this chamber of the heart. It is interesting to note that valproic acid, which exhibits selectivity for class I HDACs, did not cause adverse effects in the PAB model. The present findings suggest that, with regard to the RV, isoform-selective HDAC inhibition is safer than non-selective suppression of HDAC activity. This is evidenced by the ability of MGCD0103 to block RV apoptosis and inflammation and maintain RV contractile function in chronically hypoxic rats (Figure 4 and Table 1). Nonetheless, it should be noted that the model used for our studies (3 weeks of hypoxia in SD rats) is mild with regard to RV remodeling (Table 1), and represents a model of PH caused by interstitial lung disease and/or hypoxemia (World Health Organization [WHO] Group III PH). It will be important to extend the current findings to more severe models of PH and RV dysfunction, such as the SUGEN plus hypoxia model, to determine whether the beneficial effects of class I HDAC inhibitors are generalizable to other forms of PH, including WHO Group I idiopathic pulmonary arterial hypertension (iPAH).

The modest effect of MGCD0103 on RV hypertrophy is surprising given the prior demonstrations of anti-hypertrophic activity of HDAC inhibitors in models of LV dysfunction, but is consistent with the inability of TSA to block hypertrophy in response to
These results may reflect differential requirements for HDACs in the control of hypertrophy in the LV and RV and, more broadly, differences in signaling and transcriptional mechanisms that control growth of the two chambers. Interestingly, tadalafil reduced RV hypertrophy as efficiently as MGCD0103 despite having a more modest effect on PAP than the class I HDAC inhibitor. This finding may point to a direct role for PDE5 in the regulation of RV growth in response to chronic hypoxia.

The data presented here suggest that class I HDACs function in multiple cell types in lungs and RV as key components of pathogenic pathways that trigger increases in pulmonary vascular resistance and culminate in right-sided heart failure. Robust efforts in industry are focused on clinical development of isoform-selective HDAC inhibitors for oncology and non-oncology indications. Our results validate a role for class I HDACs in the pathogenesis of PH and justify expanded pre-clinical and clinical evaluation of class I HDAC inhibitors to determine the utility of this compound class for patients with pulmonary hypertension and RV failure.

### Novelty and Significance

**What Is Known?**

- Small molecule inhibitors of HDACs are efficacious in animal models of LV dysfunction.
- The roles of HDACs in RV remodeling are poorly understood.
- Pulmonary hypertension is associated with remodeling of the lung vasculature due to excessive proliferation of multiple cell types.

**What New Information Does This Article Contribute?**

- Isoform-selective HDAC inhibitors (class I HDAC-specific) suppress hypoxia-mediated PH.
- Efficacy of these compounds is due, in part, to suppression of cell proliferation in the lung vasculature.
- RV function is maintained in the face of class I HDAC inhibition, and cellular events associated with adverse RV remodeling are blocked by class I HDAC inhibition.

HDAC inhibitors are efficacious in models of LV failure, blocking cardiac hypertrophy and fibrosis and improving systolic and diastolic performance of the ventricle. These results have suggested unforeseen potential for this class of compounds for the treatment of heart failure. However, in a recent study, a pan-HDAC inhibitor was shown to negatively impact the RV in the setting of pressure overload. The current study was performed to address the hypothesis that selective inhibition of a subset of HDACs, rather than all HDACs, will provide a safe and efficacious means of treating PH and associated RV remodeling. In addition, since the pathogenesis of PH involves increased proliferation of cells in the pulmonary vasculature, we reasoned that the anti-proliferative action of HDAC inhibitors would suppress PH. We show for the first time that small molecules that selectively block class I HDACs (HDACs −1, −2 and −3) reduce hypoxia-induced PH in a manner that correlates with blunted medial thickening of pulmonary arteries, and reduced proliferation of SMCs in these vessels. Importantly, class I HDAC inhibition was well tolerated by the RV and associated with blockade of cellular events (e.g., myocyte apoptosis and inflammation) that contribute to adverse RV remodeling.
These findings justify expanded evaluation of class I HDAC inhibitors to determine the utility of this compound class for patients with PH and RV failure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank A. Flockton and K. Colvin for cell culture and biochemical analyses, and R. Tuder for helpful discussions.

Sources of Funding

P.A.W. was supported by a Department of Veterans Affairs Merit Award.

Non-standard Abbreviations and Acronyms

**PH** pulmonary hypertension  
**HDAC** histone deacetylase  
**PAP** pulmonary arterial pressure  
**PAAT** pulmonary artery acceleration time  
**VTI** velocity time integral

References


_Circ Res. Author manuscript; available in PMC 2013 June 14._


Figure 1. Class I HDAC inhibition in a hypoxia model of pulmonary hypertension
A. Rats were housed in hypobaric chambers and were injected i.p. with the class I HDAC inhibitor, MGCD0103 (10 mg/kg), every other day for three weeks. On days when compound was not delivered, animals were treated with vehicle control. Normoxic and hypoxic control rats were dosed with compound vehicle on a daily basis. B. Rats were weighed daily. C and D. Class I and class IIa HDAC enzymatic activity was quantified in lung and RV homogenates. E. Lung homogenates were immunoblotted for acetyl- and total α-tubulin. Lung lysates from an independent study with the pan-HDAC inhibitor, SAHA, were used as controls.
Figure 2. Class I HDAC inhibition suppresses hypoxia-dependent pulmonary hypertension
A – C, Pulmonary arterial pressure (PAP) was measured in normoxic and hypoxic rats treated with vehicle or MGCD0103 for 3 weeks. PA systolic pressure (PASP); PA pulse pressure (PAPP); mean PAP, mPAP. D, mPAP values from an independent study of tadalafil are shown. Tadalafil was dosed daily by oral gavage at a concentration of 10 mg/kg.
Figure 3. Improved hemodynamics in class I HDAC inhibitor-treated animals

A. Rats were dosed i.p. with compound vehicle or MGCD0103 (10 mg/kg) for 3 weeks. RV cardiac output was determined using an invasive pressure-volume catheter. B. Cardiac output was used to calculate total pulmonary vascular resistance. C and D. Pulmonary artery acceleration time (PAAT) and velocity time integral (VTI) were quantified using Doppler images from normoxic and hypoxic rats treated with vehicle or MGCD0103. The reduction in VTI in the hypoxia + vehicle group was significant by t-test but not ANOVA. E. Doppler images of transpulmonary blood flow. Regions of the images used to calculate PAAT and VTI are shown. Systolic notching of PA blood flow in a hypoxic rat treated with vehicle is
indicated. For all graphs, values represent mean ±SEM. *P<0.05 vs. normoxia (ANOVA); †P<0.05 vs. hypoxia plus vehicle (ANOVA); ‡P<0.05 vs. normoxia (t-test).
Figure 4. Class I HDAC inhibition suppresses multiple pathological pathways in the RV

A. Immunoblotting of class I HDAC proteins in RV lysates. C-terminus Hsp70 interacting protein (CHIP) was immunoblotted as a control. B. HDAC1 levels were quantified using a digital imager. C. RV hypertrophy was assessed by weighing ventricular chambers at the time of necropsy. D. Quantitative PCR analysis of RV brain natriuretic peptide (BNP) and alpha-skeletal actin (α-Sk-actin) mRNA levels. E. Caspase activity was measured in RV homogenates using a fluorescent substrate that is cleaved by caspase-3 and -7. For B – E, Values presented are mean +SEM. *P<0.05 vs. normoxia; #P<0.05 vs. hypoxia plus vehicle. F, RV sections from hypoxic rats stained for cleaved (active) caspase-3. Arrows indicate
caspase-positive cells. Caspase-positive cells were not detected in RVs from normoxic controls. Scale bar = 10 µm. G, Cytokine protein levels in RV homogenates. For each group, RV protein from four independent animals was pooled prior to analysis.
Figure 5. A second class I HDAC inhibitor suppresses hypoxia-dependent pulmonary hypertension and RV hypertrophy
Rats were housed in hypobaric chambers and were injected with the class I HDAC inhibitor, MS-275 (3 mg/kg), every other day for three weeks. On days when compound was not delivered, animals were treated with vehicle control. Normoxic and hypoxic control rats were dosed with compound vehicle on a daily basis. MS-275 reduced PASP (A), PAPP (B) and RV hypertrophy (C). For all graphs, values represent mean +SEM. *P<0.05 vs. normoxia (ANOVA); #P<0.05 vs. hypoxia plus vehicle (ANOVA).
Figure 6. MGCD0103 is not an acute vasodilator

A. Rat pulmonary artery strips (1.5 mm X 200 µm) were hung on a “bubble plate” between two tungsten wires. One wire was fixed and the other attached to a force transducer. Intact strips were stimulated with high potassium (KES). MGCD0103 had no effect on the potassium-mediated contraction at any concentration (10–300 nM). However, when strips were washed with normal extracellular solution (NES) and contracted again with potassium, sodium nitroprusside (SNP, 1 µM) effectively relaxed the vessel. 

B. Rats were maintained in hypobaric chambers for 3 weeks. On the final day of the study, animals received a single injection of MGDC0103 (10 mg/kg) 2 or 20 hours prior to measuring PAP. Acute administration of MGCD0103 failed to lower hypoxia-induced increases in PASP or PAPP.
Figure 7. Class I HDAC inhibition suppresses hypoxia-induced pulmonary arterial medial thickening

A, Images of hematoxylin and eosin- (upper panel) and proliferating cell nuclear antigen (PCNA)-stained lung sections. PCNA-stained sections were counterstained with hematoxylin. Scale bar = 10 µm. B, Medial thickness of pulmonary arteries between 50 and 250 µm in diameter. Values are presented as mean ±SEM. *P<0.05 vs. normoxia; #P<0.05 vs. hypoxia plus vehicle. C, Rat PASMCs were cultured in the presence of FBS (10%) with the indicated concentrations of MGCD0103. D, Rat PASMCs were grown under normoxic or hypoxic conditions for 48 hours in the absence or presence of MGCD0103 (500 nM). Protein lysates from these cells were immunoblotted with antibodies for FoxO3a, phospho-
FoxO3a (P-Thr-32), p27 or α-tubulin. E, Homogenates of lungs (n = 4 lungs/condition, pooled) were immunoblotted for FoxO3a, p27 or α-tubulin.
Invasive hemodynamics

Table 1

Invasive hemodynamic assessments of RV function were performed with a Scisense pressure-volume system.

<table>
<thead>
<tr>
<th></th>
<th>Normoxia + Vehicle</th>
<th>Hypoxia + Vehicle</th>
<th>Hypoxia + MGCD0103</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>357 ± 7</td>
<td>343 ± 5</td>
<td>361 ± 5</td>
</tr>
<tr>
<td>RV End-systolic Pressure (mmHg)</td>
<td>30 ± 7</td>
<td>60 ± 10*</td>
<td>47 ± 8 *#</td>
</tr>
<tr>
<td>RV End-diastolic Pressure (mmHg)</td>
<td>4 ± 1</td>
<td>6 ± 3</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Stroke work (mmHg µl)</td>
<td>4569 ± 1034</td>
<td>8634 ± 2048 #</td>
<td>8034 ± 2306 #</td>
</tr>
<tr>
<td>Cardiac Output (ml/min)</td>
<td>82.6 ± 6.4</td>
<td>82.2 ± 11.7</td>
<td>83.7 ± 9.3</td>
</tr>
<tr>
<td>Ejection Fraction (%)</td>
<td>80.6 ± 4.6</td>
<td>71.8 ± 4.2 *</td>
<td>70.7 ± 6.3 *</td>
</tr>
<tr>
<td>dP/dt max (mmHg/s)</td>
<td>1634 ± 295</td>
<td>2638 ± 385 *</td>
<td>2804 ± 377 *</td>
</tr>
<tr>
<td>dP/dt min (mmHg/s)</td>
<td>−1586 ± 478</td>
<td>−2822 ± 684 *</td>
<td>−2786 ± 394 *</td>
</tr>
<tr>
<td>Preload recruitable stroke work (mmHg)</td>
<td>22.4 ± 7.3</td>
<td>30.0 ± 10.0 *</td>
<td>35.4 ± 15.4 *</td>
</tr>
<tr>
<td>Ea (mmHg/µl)</td>
<td>0.12 ± 0.04</td>
<td>0.26 ± 0.07 *</td>
<td>0.21 ± 0.03 *</td>
</tr>
</tbody>
</table>

Values represent averages +/- standard deviation. Normoxia (n = 6), hypoxia (n = 10), hypoxia + MGCD0103 (n = 9).

*P<0.05 vs. normoxia;

#P<0.05 vs. hypoxia plus vehicle.