Celastrol Attenuates the Remodeling of Pulmonary Vascular and Right Ventricular in Monocrotaline-Induced Pulmonary Arterial Hypertension in Rats

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Abstract

Purpose

Pulmonary arterial hypertension (PAH) is a progressive angio-proliferative disease with high morbidity and mortality. Although the histopathology is well described, its therapeutic effect is still unsatisfactory. This study investigated the treatment of celastrol on PAH rats right ventricular (RV) dysfunction, RV remodeling and pulmonary vascular remodeling and its possible mechanisms.

Methods

PAH in rats was induced by monocrotaline (MCT) single subcutaneously injection. After daily delivery of Celastrol (1mg/kg) or vehicle via intraperitoneal injection for 4 weeks, the effects of celastrol on RV function, fibrosis, and pulmonary vascular remodeling were assessed. Macrophage infiltration, expression of inflammatory cytokines, including MCP-1, IL-1 β , IL-6, IL-10 and the expression of NF- κ B signaling pathway-associated protein, I κ B α , p-IKK α / β and p65 were further detected. Finally, the effect of celastrol on human pulmonary artery smooth cells (HPASMCs) proliferation under hypoxia was studied in vitro.

Results

Rats with PAH had degressive RV function, increased RV fibrosis and pulmonary arteries with interstitial thickening and prominent media hypertrophy. Treatment with celastrol improved RV function, attenuated RV fibrosis and pulmonary vascular remodeling. Significantly decreased macrophage infiltration, lower level of pro-inflammatory cytokines, higher level of anti-inflammatory cytokine and inhibited NF- κ B signaling pathway were observed in lung tissues of rats with celastrol treatment. Moreover, celastrol significantly suppressed the proliferation of HPASMCs under hypoxia.

Conclusions

We showed that, in rats with PAH, celastrol could improve RV function, attenuate RV and pulmonary vascular remodeling and the proliferation of HPASMCs under hypoxia. The suppression of NF- κ B signaling pathway may be a part of the protective mechanism.

Introduction

Although Pulmonary arterial hypertension(PAH) is a rare disease, the incidence of PAH awfully ranges from 2.0 to 7.6 cases per million adults per year, and its prevalence varies from 11 to 26 cases per million adults[1]. PAH is characterized by progressive pulmonary arterial remodeling. Arteriopathy in the small to medium-sized distal pulmonary arteries is associated with arterial muscularization, concentric intimal thickening, and the formation of plexiform lesions which results in increased pulmonary vascular resistance, leading to right ventricle (RV) overload[2]. Initially, the RV adapts to the increased afterload by hypertrophying and increasing contractility, but as the disease progresses, RV fails. Therefore, RV failure is the main cause of death in patients with PAH. Despite the improvements in treatment options, the

overall survival of PAH still remains unsatisfactory[3]. During the past two decades, several effective medications have been discovered, including prostacyclin analogs and derivatives, endothelin receptor antagonists, phosphodiesterase type 5(PDE5) inhibitors, and an soluble guanylate cyclase(sGC) stimulator[4]. However, there is still no treatment available that targets both the pulmonary arteriole and the RV, and no satisfactory medicine can prevent or reverse disease progression.

Even though the pathogenesis of the PAH is largely unknown, there is no doubt that early and persistent inflammation plays an indispensable role in the development and progress of PAH. Inflammatory cells such as macrophages, T cells and B cells infiltrate the plexiform lesions in patients with advanced PAH[5]. Moreover, levels of several inflammation cytokines and cytokines receptors are changed in PAH lungs, and some of them are correlated with the disease severity and prognosis[6]. Among the proinflammatory signaling pathways, nuclear factor- κ B (NF- κ B) plays an important role [7, 8].

Considering the critical role of inflammation in PAH and the anti-inflammatory effects of celastrol, we therefore evaluated whether celastrol has protective effects against MCT-induced PAH.

Methods

Animals and in vivo treatment

To limit the potential gender differences, we selected only male rats. Young male Sprague-Dawley (220-250 g, Laboratory Animal Center of Sun Yat-sen University, Guangzhou, China) were studied 4 weeks after a single subcutaneously injection of MCT (60 mg/kg; Sigma, USA) or vehicle[18]. From the first day, MCT-injected rats were randomly divided into two groups. Rats from one group received daily intraperitoneal injection of celastrol (1mg/kg/day, TargetMol, Shanghai, China) for 4 weeks and vehicle [0.9% dimethylsulfoxide+2% Tween80+97.1% saline] in the same volume for another group. Rats were maintained on standard laboratory rat chow and water ad libitum and kept on a 12-h/12-h light–dark cycle.

Echocardiography

Rats were anesthetized with continuous isoflurane inhalation (1.5-3%) and kept with spontaneous respiration. Transthoracic echocardiography was performed by a 25 MHz linear array transducer (Vevo 2100, VisualSonics, Toronto, Canada). Short axis M-mode recordings were obtained to measure left ventricle ejection fraction (LVEF). The pulsed-wave doppler recording at the right ventricular overflow tract was used to measure pulmonary acceleration time (PAT), peak ejection time (PET) and pulmonary artery velocity time integral (PA-VTI). Tricuspid annular plane systolic excursion (TAPSE) was measured by using M-mode across the tricuspid valve annulus at the RV free wall. TAPSE was determined by measuring the excursion of the tricuspid annulus from its highest position to the peak descent during ventricular systole. Analyses were performed with observers blinded to the source of the images.

RV catheterization and hypertrophy index

After Echocardiography, terminal invasive hemodynamic measurements were performed to confirm RV pressure via RV catheterization. The rats were anaesthetized with pentobarbital sodium (40mg/kg) and fixed on plank, then the right jugular vein was isolated and freed for intubation. The PE-50 tube was filled with heparin saline, connected to a pressure sensor (Techman, Chengdu, China) and inserted into the right external jugular vein. The appearance of the ventricular pressure wave indicated that the catheter reached the RV. Then the RV pressure (RVP) was recorded and the systolic pressure (RVSP) was analyzed. After the RVSP was measured, the chest was quickly opened to harvest the heart and lungs of rats. The left and right atrium and blood vessels along the junction of the atrioventricular compartment were cut. Then the RV, left ventricle (LV) and interventricular septum (IVS) were separated and measured separately. The RV hypertrophy index was calculated as $[RV/(LV + IVS)]$.

Masson staining and hematoxylin-eosin (H&E) staining

After hemodynamic measurements and blood withdrawals, heart and lungs were excised and harvested for fibrosis, morphometric and histologic analysis. The heart and middle lobe of the right lung were dissected and fixed in 4% paraformaldehyde for 24h, then embedded in paraffin and sectioned. Heart sections were stained with Masson's trichrome and lung sections were stained with hematoxylin and eosin (H&E). A light microscope (Carl Zeiss, Jena, Germany) was used for overall histological assessment. The fibrosis of RV was assessed by Image-Pro Plus software (Version 6.0, Media Cybernetics, Silver Springs, MD, USA). Pulmonary arterial wall thickness (WT) was calculated by the following formula: $WT (\%) = \frac{\text{area}_{\text{ext}} - \text{area}_{\text{int}}}{\text{area}_{\text{ext}}} \times 100$, where area_{ext} and area_{int} are areas bounded by external and internal elastic lamina, respectively.

Immunohistochemistry

Western Blot

Flash-frozen inferior lobe of right lungs were homogenized in radioimmunoprecipitation assay buffer supplemented with Halt protease and phosphatase inhibitor cocktail (Thermo Fisher scientific, Waltham, MA, USA). Protein content was determined by using a BCA protein assay (Thermo Fisher scientific, Waltham, MA, USA). Equivalent amount of protein was resolved by SDS-polyacrylamide gel electrophoresis (PAGE) (10%) and transferred to polyvinylidene difluoride membranes. After being blocked for 1 h in 5% nonfat dry milk and tris-buffered saline, the membrane was incubated with the desired primary antibody for 8 h in 4°C. The membrane was then treated with appropriate horseradish peroxidase conjugated secondary antibody (Cell Signaling Technology, Danvers, MA, USA), and the immuno-reactive bands were detected by chemiluminescence (ECL) reagents (Merck Millipore, Billerica, MA, USA). Specific antibodies for TGF- β 1 (sc-130348, Santa Cruz, CA, USA), I κ B α (4814S, Cell Signaling Technology, Danvers, MA, USA), phosphorylated IKK α / β (2697S, Cell Signaling Technology, Danvers, MA, USA), p65 (sc-8008, Santa Cruz, CA, USA), GAPDH (60004-1-Ig, Proteintech, Chicago, IL, USA) were used in immunoblot assay. For analysis, the expression of target proteins was normalized to GAPDH.

Quantitative Real-time RCR

The superior lobe of right lungs, isolated from the rats, were homogenized and total RNA was purified with TRIzol reagent (Thermo Fisher scientific, Waltham, MA, USA) according to the manufacturer's instructions. One thousand nanograms of RNA was reverse-transcribed to cDNA by using the PrimeScript™ RT reagent Kit (RR037A, kara, Tokyo, Japan). PCR primers were designed and synthesized by Invitrogen (Thermo Fisher scientific, Waltham, MA, USA). The following specific primers were generated and used: for Collagen I, forward primer: GTACATCAGCCCAAACCCCA, reverse primer: TCGCTTCCATACTCGAACTGG; for Collagen III, forward primer: TGGTGGCTTTCAGTTCAGCTA, reverse primer: ATTGCCATTGGCCTGATCCA; for TGF-β1, forward primer: CTGCTGACCCCCACTGATAC, reverse primer: AGCCCTGTATTCCGTCTCCT; for monocyte chemotactic protein 1 (MCP-1), forward primer: TGTCTCAGCCAGATGCAGTT, reverse primer: CAGCCGACTCATTGGGATCA; for IL-1β, forward primer: TAGCAGCTTTCGACAGTGAGG, reverse primer: CTCCACGGGCAAGACATAGG; for IL-6, forward primer: CATTCTGTCTCGAGCCCACC, reverse primer: GCTGGAAGTCTCTTGCGGAG; for IL-10, forward primer: CCTGGTAGAAGTGATGCCCC, reverse primer: GACACCTTTGTCTTGAGCTTAT; for GAPDH, forward primer: CGCTAACATCAAATGGGGTG, reverse primer: CGCTAACATCAAATGGGGTG. Quantitative PCR was performed using TB Green® Premix Ex Taq™ II (RR820A, Takara, Tokyo, Japan) and assayed in duplicate, according to the manufacturer's instructions in a BioRad CFX96 PCR system (Bio-Rad, Hercules, CA, USA). For analysis, the expression of target genes was normalized to GAPDH.

EdU proliferation assay

Cell proliferation was studied with EdU Cell Proliferation Assay Kit (EdU-488, Beyotime, Shanghai, China), according to the manufacturer's protocol. 1.8×10^4 human pulmonary artery smooth cells (HPASMCs, purchased from ScienCell, Carlsbad, CA, USA) were seeded into 24-well plates per well and cultured overnight. Then the medium was replaced and 0.5μM or 1.0μM celastrol was added to the medium, and HPASMCs were exposed to hypoxic (2% oxygen) or normoxia (21% oxygen) conditions at 37°C for 36h. After that, 10μM EdU was added to the cells. At the end of the 4-hour incubation, the medium was removed, and the cells were fixed in 4% paraformaldehyde for 15min at room temperature. After the aspiration of the fixing solution, the cells were stained with the BeyoClick™ EdU Cell Proliferation Kit with Alexa Fluor 488 and DAPI (nuclear stain). Then the results were visualized through a fluorescence microscope (Leica, Wetzlar, Germany). The results were quantified by using Image-Pro Plus software (Version 6.0, Media Cybernetics, Silver Springs, MD, USA).

Cell viability assay

Cell viability was assessed by using cell counting kit-8 (CCK-8, Beyotime, Shanghai, China). In brief, HPASMCs were seeded into 96-well plates at a concentration of 6×10^3 cells/well and cultured for 24h. Then the medium was replaced and 0.5μM or 1.0μM celastrol was added to the medium, and HPASMCs were exposed to hypoxic (2% oxygen) or normoxia (21% oxygen) conditions at 37°C for 36h. After the exposure and the replacement of the medium, 10μL CCK-8 solution was added to each well and incubated at 37°C for another 3 h. Optical density value at a wavelength of 450 nm was determined by using a microplate reader (Thermo Fisher scientific, Waltham, MA, USA).

Statistical analyses

All data was presented as mean±SEM. All statistical analyses were performed by using GraphPad Prism software (Version 8, La Jolla, CA, USA). Comparisons among groups were assessed with one-way ANOVA followed by Tukey's post hoc test. Differences were considered statistically significant if $p < 0.05$.

Results

1. Celastrol improves RV function and reduces RVSP in PAH rats

Four weeks after MCT injection, PAH rats models were established. Reduced PAT, PAT/PET and TAPSE were observed compared with the normal rats, which confirmed the RV dysfunction. Treatment with celastrol significantly improved RV function of PAH rats relative to that with the vehicle treatment (Fig. 1a, 1c and 1e). The PAT-VTI of vehicle group decreased significantly compared to the normal group. Although the celastrol group increased the PAT-VTI compared to the vehicle group, there was no statistical difference (Fig. 1d). Furthermore, four rats died before the end of study. All of them were from the vehicle group. Necropsy of the dead animals did not reveal a clear cause of death. However, celastrol treatment completely prevented mortality (Fig. 1f). As we expected, LVEF had no significant change in any of the groups (Fig. 1g), meaning neither MCT nor celastrol had any effect on LV function. To explore the role of celastrol in the development of PAH, we examined RVSP with a pressure catheter which can represent pulmonary artery pressure more accurately. As is shown in Fig. 1h, celastrol dramatically attenuated the PAH by reducing RVSP to 83.82%.

2. Celastrol alleviates RV hypertrophy and fibrosis in PAH rats

Due to the increased RV pressure in PAH rats, the RV was remodeled and enlarged, which was shown as a higher RV/(LV+IVS) ratios. However, celastrol group had a significantly lower RV/(LV+IVS) ratio compared with vehicle group (Fig. 2a). Using Masson staining, we found a 2.82-fold increase in the volume fraction of fibrosis in the RV in vehicle rats compared with normal rats. Treatment with celastrol significantly reduced RV fibrosis (Fig. 2c). Likewise, the mRNA expressions of fibrosis-related molecules in RV, collagen I and collagen III increased in PAH rats. Celastrol treatment significantly reduced their expressions (Fig. 2d and 2e).

3. Celastrol attenuates pulmonary vascular remodeling

MCT injection induced remodeling of the pulmonary vascular evident by increasing wall thickness of the smaller pulmonary arteries (PA wall thickness) with an outer diameter between 50 and 100µm. Celastrol treatment led to a modest suppression of MCT-induced PA wall thickness (Fig. 3a).

Immunohistochemistry of α-SMA showed increased pulmonary arteries muscularization in pulmonary arteries with an outer diameter between 50 and 100µm in vehicle rats, whereas were weakened by celastrol treatment. Furthermore, celastrol significantly reduced the expression of TGF-β1 in the superior lobe of right lung at the mRNA and protein level compared with vehicle rats (Fig. 3e and 3g).

4. Celastrol inhibits inflammatory in lungs

Previous studies have demonstrated that elevated macrophage infiltration and enhanced levels of inflammatory cytokines are correlated with the severity of PAH[6]. We therefore wondered whether celastrol treatment affected the infiltration of macrophage and the production of inflammatory cytokines in vivo. As is shown in Fig. 4a and b, celastrol treatment markedly decreased the number of macrophages in the lungs of the PAH rats compared with the vehicle treated PAH rats. The RT-qPCR revealed that the expression levels of several proinflammatory cytokines in lungs, including MCP-1, IL-1 β and IL-6, significantly increased in the vehicle group compared with those in the normal group, but they were significantly inhibited by celastrol treatment (Fig. 4c-e). Celastrol further remarkably increased the expression of IL-10 in the lungs of rats with PAH, which is an anti-inflammatory factor (Fig. 4f).

5. Celastrol inhibits NF-kB signaling pathway in lungs

Given that the NF-kB signaling pathway plays a crucial role in PAH initiation and progression[7, 8], we next tested whether celastrol inhibited inflammation through the NF-kB signaling pathway. In PAH rats, lungs NF-kB signaling pathway was active (Fig. 5a-d), shown up as increased degradation of I κ B α , phosphorylation of IKK α / β and expression of p65. However, Celastrol treatment significantly antagonized the effects of MCT in rats' lungs.

6. Celastrol inhibits the proliferation of HPASMCs in response to hypoxia

The proliferation of PSMCs contributes to the progression of vascular remodeling which leads to irreversible progression of PAH[19]. We thus examined the effect of celastrol treatment on the proliferative potential of cultured HPASMCs. EdU proliferation assay showed that hypoxia promoted HPASMCs proliferation, but celastrol exhibited anti-proliferative effect on HPASMCs with both low and high concentrations (0.5 μ M and 1.0 μ M) under hypoxia (Fig. 5f). However, CCK-8 assay showed that low concentration celastrol had a tendency to enhance the cell activity of HPASMCs under hypoxia without a statistical difference, but high concentration celastrol had a remarkable inhibit effect(Fig. 5g).

Discussion

Using a universally adopted model of PAH, we found that treatment with celastrol for 4 weeks significantly improved RV function and decreased pulmonary artery pressure. We also demonstrated that these improvements were associated with decreased RV and pulmonary vascular remodeling, reduced lung macrophage infiltration and inflammation and decreased collagen content in RV. Furthermore, we found that celastrol inhibits the proliferation of HPASMCs under hypoxia in vitro. Finally, we found that the decreased remodeling of pulmonary vascular may be related to the inhibition of NF-kB signaling pathway.

Recently, more and more evidences have indicated that macrophages play an important role in pulmonary vascular remodeling of PAH. Macrophage infiltration is not only a characteristic hallmark, but

also a critical component of pulmonary vascular remodeling associated with PAH[21]. Abid S et al[22] found that macrophages and PSMCs communicated to promote PSMCs proliferation via CCR2(receptor of MCP-1) and CCR5, and targeting CCR2, CCR5 or both can prevent or reverse PAH in mice. In a rat arthritis model, Celastrol was found to significantly decrease the number of CD68⁺ macrophages and the overall inflammatory cellularity in the arthritic synovial tissue without side effects[23]. Consistent with these findings, our results suggested that celastrol therapy significantly reduced pulmonary macrophage infiltration and decreased the mRNA expression of MCP-1 induced by MCT, which may partly explain the lighter pulmonary vascular remodeling in celastrol rats. Along with macrophage infiltration, the up-regulated expression of pro-inflammatory cytokines and down-regulated expression of anti-inflammatory cytokines are also characteristics of PAH and further promote its development[24]. For instance, elevated IL-6 levels exhibited critical roles in a mice hypoxia-induced PAH by promoting the polarization of macrophage polarization in the lungs[25]. In this study, we found that celastrol treatment significantly reduced the expression of pro-inflammation cytokines IL-1 β , IL-6, and TGF- β 1 in the lungs of rats with PAH, and increased the expression of anti-inflammation cytokines IL-10.

NF- κ B signaling pathway regulates the expression of wide a variety of genes involved in cell proliferation, inflammation, survival, migration and angiogenesis[26, 27]. Thus NF- κ B signaling pathway has long been considered the holy grail as a target for new anti-inflammatory drugs[28]. Sethi G et al [29] found that celastrol could apparently inhibit the TNF- α induced activation of I κ Ba kinase, I κ Ba phosphorylation, I κ Ba degradation, p65 nuclear translocation and phosphorylation, and NF- κ B mediated reporter gene expression. Importantly, celastrol was also found to inhibit IKK activity and the constitutively active IKK β activity in a dose dependent manner without directly suppressing the DNA binding ability of NF- κ B[30]. Consistency with previous studies, our study confirmed that celastrol can indeed inhibit the activation of the NF- κ B signaling pathway in rats lungs with PAH. The inhibition of NF- κ B signaling pathway well explained the reduction of lung inflammation and less severe pulmonary vascular remodeling in celastrol treated rats. Several published reports within the last decade showed that activation of NF- κ B promotes cell survival and proliferation and downregulation of NF- κ B sensitizes the cells to apoptosis induction[31, 32]. However, Celastrol reportedly has anti-cancer effects both in vitro and in vivo[33]. For instance, Guo et al recently found that celastrol induces gastric cancer cell death via necroptosis[34]. Due to the unrestricted proliferation and apoptosis resistant of PSMCs, PAH is somewhat similar to tumor disease[1]. Therefore, it is very important to inhibit the proliferation of PSMC to prevent the progression of PAH. It is consistent with Kurosawa R et al[35] research, we demonstrated that low and high concentration of celastrol significantly inhibited the proliferation of HPASMCs under hypoxia using EdU proliferation assay. To our surprise, CCK-8 assay showed that low concentration of celastrol tended to promote promoted the cell viability of HPASMCs under hypoxia. Der Sarkissian S et al[36] had previously shown that celastrol could confers cardio-protection against ischemic injury in an animal model of MI in vivo and improves cell viability under hypoxia in vitro via reactive oxygen species (ROS) signal. Additionally, considering the optical density value at a wavelength of 450 nm of CCK-8 kit is depending on cell's mitochondria, we hypothesized that low concentration of celastrol possibly can protect HPASMCs for oxidative stress under hypoxia without adequate ability to inhibit proliferation. Thus, the

precise effect of different concentrations of celastrol on HPASMCs and its mechanism deserve further study.

The safety of drugs is an important indicator of future clinical use. Although we did not explore the safety of celastrol in vivo, Zhao and Gong et al [37, 38] had already proved that small doses of celastrol was safe in mice and rats. Combined with our founding that low concentration of celastrol possibly promoted the cell viability of HPASMCs under hypoxia, which means that celastrol could one day be used to treat patients with PAH safely.

In summary, we observed favorable changes in RV systolic pressure, RV hypertrophy, and RV function in celastrol treated animals in a model of PAH induced by MCT. Our results validate celastrol as a promising compound for the treatment of inflammation and provide relevant insights into the usage of celastrol as a future drug for PAH. It would be interesting to extend this knowledge by studying the anti-inflammatory and anti-proliferation of PSMCs properties of celastrol in vivo using different animal models of PAH.

Declarations

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Not applicable

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Conflict of interest

The authors declare no competing interests.

Availability of data and material

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' Contributions

All authors contributed to the study conception and design. Huayang Li conceived the study and was responsible for the data acquisition, analysis, and interpretation and wrote the first draft of the paper. Quan Liu, Yuan Yue carried out the experiments and contributed to the data acquisition. Shunjun Wang, Suiqing Huang, Lin Huang and Li Luo helped with the data analysis, manuscript composition, and

proofreading. Yitao Zhang contributed substantially to the conception and design of the study and helped with the data analysis and interpretation. Zhongkai Wu acquired funding, supervised the study and edited the manuscript. All authors read, discussed, commented on and approved the final version of the manuscript.

Ethics approval

This protocol was approved by the Institutional Animal Use and Care Committee of Sun Yat-sen University (2020000093). All experiments were conducted in strict accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (USA National Institutes of Health publication No. 80-23, revised 1996) and the institutional policies of Sun Yat-sen University.

Consent to Participate

Not applicable.

Consent for Publication

The manuscript has been approved by all the authors for publication.

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Figures

Figure 1

Celastrol treatment improves RV function and reduces RVSP in PAH rats. Quantification of PAT(a), PET(b), PAT/PET(c), PA-VTI(d), TAPSE(e), LVEF(g), RVSP(h) four weeks after MCT injection (n=9 for each group); (f) Kaplan-Meier survival curves in the different groups (n=10 for the normal group, n=13 for the vehicle group, n=12 for the celastrol group). *p<0.05, **p<0.01, ***p<0.001.

Figure 2

Celastrol alleviates RV hypertrophy and fibrosis in PAH rats (a) RV hypertrophy index; (b) Histopathological features of collagen deposition based on the Masson staining of heart sections (40×); (c) Quantification of the Masson staining; (d), (e) RV mRNA expression of collagen I and collagen III. n=8 for each group. *p<0.05, **p<0.01, ***p<0.001.

Figure 3

Celastrol attenuates pulmonary vascular remodeling (a) Quantification of the medial wall thickness of the small pulmonary arteries with an outer diameter between 50 and 100μm. (b) Photomicrographs of lung tissue sections stained with H&E (99×); (c) Quantification of the arteries muscularization of the small pulmonary arteries with an outer diameter between 50 and 100μm; (d) Expression of α-SMA in lungs evaluated with immunohistochemistry staining (99×); (e) mRNA expression of TGF-β1 in lung tissues; (f)

Western blots of TGF- β 1 in lung tissues; (g) Quantification of the TGF- β 1 expression in lung tissues. n=8 for each group. *p<0.05, **p<0.01, ***p<0.001.

Figure 4

Celastrol inhibits inflammatory in lungs of PAH rats (a) Quantification of CD68 positive cells per view; (b) Representative images of CD68 positive cells in lung tissues (20x); (c), (d), (e) and (f) mRNA expression of MCP-1, IL-1 β , IL-6 and IL-10 in lung tissues. n=8 for each group. *p<0.05, **p<0.01, ***p<0.001.

Figure 5

Celastrol inhibits NF-κB signaling pathway in lungs of PAH rats and the proliferation of HPASMCs in response to hypoxia in vitro (a) Western blots of IκBα, p-IKKα/β and p-65 in lungs; (b), (c) and (d) quantification of the IκBα, p-IKKα/β and p-65 expression in lungs. n=8 for each group. (e) Representative images of the EdU assay of HPASMCs in vitro (100×); (f) and (g) quantification of the EdU assay and

CCK-8 assay of HPASMCs (n=3, biological replicates; 4 wells each time for EdU assay, 3wells each time for CCK-8 assay). *p<0.05, **p<0.01, ***p<0.001.