The potential of asiaticoside for TGF-β1/Smad signaling inhibition in prevention and progression of hypoxia-induced pulmonary hypertension

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Aims: Asiaticoside (AS) is a saponin monomer extracted from the medicinal plant Centella asiatica, which has a variety of biological effects. We intended to investigate the effects of asiaticoside on a hypoxia-induced pulmonary hypertension (HPH) rat model and examine the possible effects of asiaticoside on TGF-β1/Smad signaling in vitro.

Main methods: The rat HPH model was established by hypoxic exposure and asiaticoside was administered for four weeks. Parameters including the mean pulmonary artery pressure (mPAP), the right ventricular hypertrophy (RVH) and the percentage of medial wall thickness were used to evaluate the development of HPH. TGF-β1, TGF-β1 receptor, Smad2/3 and phospho-Smad2/3 expressions were detected and the proliferation, migration and apoptosis of pulmonary arterial smooth muscle cells (PASMCs) adjusted by asiaticoside under the hypoxic condition were evaluated.

Key findings: Our data indicate that asiaticoside attenuated pulmonary hypertension, pulmonary vascular remodeling and RV hypertrophy in HPH rats, which was probably mediated by restraining the hypoxia-induced over active TGF-β1/Smad2/3 signaling and inhibiting the proliferation by inducing apoptosis of the PASMCs.

Significance: Given the preventative potential in animal models and in vitro, we propose asiaticoside as a promising protective treatment in HPH.

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1. Introduction

Pulmonary arterial hypertension (PAH) is an unexplained progressive disorder characterized by a sustained increase in pulmonary artery pressure, pulmonary vascular remodeling, and right ventricular hypertrophy, which affects the quality of life and ultimately leads to premature death [13]. Vascular proliferative remodeling is thought to play a central role in the morbidity and progression of the disease [25]. Insufficient ability to reverse vascular remodeling is an important cause for the failure of treatment in PAH. As a pivotal pathologic process that results in irreversible PAH, pulmonary vascular remodeling has turned into an important target for therapeutic intervention [4,34] and increasingly studies have focused on reversing the proliferation and fibrosis of pulmonary arteries.

Dysregulated TGF-β1 signaling contributes pulmonary artery remodeling and has been suggested to promote PAH [5,10,22], especially through enhanced cellular proliferation [2]. TGF-β1 exerts its effect via the TGF-β receptors, such as TGF-βRI and TGF-βRII. Hypoxia-induced pulmonary artery remodeling was greatly attenuated in a transgenic mouse model that expresses an inducible dominant-negative mutation of the TGF-βRII receptor (DNTGF-βRII mouse) [2]. Excessive TGF-β1 signaling can initiate profibrotic gene expression, which occurs via Smad2/3 proteins. The phosphorylation of Smad2 increases in plexiform lesions of patients with PAH [30]. The hypoxia-induced over-expression of TGF-β1 was proposed to increase the expression of NOX4 and oxygen free radicals through Smad2/3 signal pathways and regulate the proliferation of PASMCs, responsible for pulmonary artery reconstruction [32, 16].

Asiaticoside (AS) is a monomer extracted from the medicinal plant Centella asiatica. Asiaticoside has a variety of biological actions including anti-inflammatory [37] and anti-hepatofibrotic effects [11], as well as serving as an inhibitor of melanogenesis [17], a neuroprotector against transient cerebral ischemia and reperfusion [7] and an inducer of apoptosis of tumor cells [11,15]. In particular, research has revealed that asiaticoside functioned effectively in preventing keloids and hypertrophic scars by suppressing the TGF-β1 induced expression of collagen [33]. The involvement of activated TGF-β1 signaling in the pathogenesis...
of PAH inspired the hypothesis that AS may also suppress pulmonary vascular remodeling by inhibiting activated TGF-β1 signaling in PAH.

To test our hypothesis, we first investigated the effects of asiaticoside on mean pulmonary arterial pressure, right ventricle hypertrophy (RVH) and morphological changes in a hypoxia-induced pulmonary hypertension (HPH) rat model. Subsequently, we examined the possible action mechanisms of asiaticoside by investigating TGF-β1/Smad signaling in the HPH rat model. Finally, we examined the effects of asiaticoside on the pulmonary arterial smooth muscle cells (PASMCs) of rats to illustrate the effect of asiaticoside on PAH at a cellular level.

Fig. 1. Effect of asiaticoside on hypoxia induced pulmonary hypertension. Rats in hypoxia + control group and hypoxia + AS group exposed to hypoxia for 4 weeks; asiaticoside was given at 50 mg/kg daily by intragastric administration. (A) Mean pulmonary artery pressure (mPAP), (B) carotid arterial pressure (CAP), (C) RV/body weight, and (D) right ventricular hypertrophy (RV / LV + S). Data are presented as mean ± SD. (E) Demonstrative traces of mPAP of each group of animals. Data analyzed by one-way standard ANOVA, comparing all groups pairwise. Compared with hypoxia + control group, #P < 0.05 and ##P < 0.01; compared with normoxia + control group, *P < 0.05 and **P < 0.01, n = 10.
2. Materials and methods

2.1. Animal treatment

Animal experiments were approved by the Institutional Animal Ethics Committee for Experimentation on Animals of Wenzhou Medical University and complied with the Animal Management Rule of the Ministry of Health, China and the US National Institutes of Health Guide. Experiments were performed with adult male Sprague–Dawley rats with a body weight of 200–250 g (SLAC Laboratory Animal Ltd., Co. Shanghai, China). Hypoxia-induced PAH was developed by keeping rats in a sealed but ventilated hypoxic chamber (9% O2; YPC-160D, Changsha Huaxiao Electronic Technology Co., Ltd., China) for 28 days as described previously [28]. To determine the effect of asiaticoside on the development of pulmonary hypertension, rats received asiaticoside (50 mg/kg) or vehicle (normal saline) daily through intragastric administration during a 4-week period of hypoxia exposure. A normoxic rat also received vehicle or asiaticoside administration as a control.

2.2. Examination of pulmonary artery pressure and right ventricular hypertrophy

Four weeks after hypoxic exposure, rats were weighed and anesthetized with sodium pentobarbital by intraperitoneal injection (35 mg/kg). Invasive hemodynamic measurements, including mean pulmonary arterial pressure and mean carotid arterial pressure (mCAP) were measured as described previously [24]. A micro-catheter was inserted through the jugular vein into the pulmonary artery, which was connected to a transducer (Powerlab 8 passages electrophysiograph, ADI, Australia). With the rat fully conscious, allowing sufficient time for recovery from the effect of anesthesia, the hemodynamic signals were saved and analyzed with the digital acquisition system. Pulmonary arterial blood pressure (PAP) was recorded on a physiologic recorder (Powerlab, Australia), and mean PAP calculated. At the end of the measurement, the right ventricular (RV) wall was separated from the left ventricular (LV) wall and the interventricular septum (S) and weighed. An index of right ventricular hypertrophy (RVH) was calculated by the right ventricle to left ventricle plus septum ratio; RV / LV + S, as described previously [6].

2.3. Histological studies and medial wall thickness

Excised lung tissue, processed for light microscopic observation, was fixed in 4% paraformaldehyde, chopped into small pieces, embedded in paraffin, and sectioned into 5-μm slices. Hematoxylin and eosin (HE) staining and elastic van Gieson (EVG) staining were performed for each rat. Transverse sections of arterioles, stained using the elastic van Gieson technique, were performed to calculate the medial wall thickness, as described previously [38]. The images of the lung tissue and pulmonary arterioles were captured with a digital microscope camera (OLYMPUS BX51 microscope, Olympus Co., Japan). The percent medial wall thickness was determined by Image-ProPlus 6.0.
2.4. Culture and identification of rat PASMCs

The pulmonary artery from male Sprague-Dawley rats (200–250 g) was removed under sterile conditions. Rat PASMCs were isolated from pulmonary arterial vessels, according to a previously reported protocol [29]. Cells were isolated from one rat in any independent experiment, and then we repeated experiments with different rats several times. Cells were identified as smooth muscle cells (SMCs) if they presented a typical “hill and valley” growth pattern and positive reaction with antibodies against SMA by immunofluorescence. For immunofluorescence, cells were incubated with an anti-αSMA rabbit antibody (1:100, Abcam) and then exposed to antirabbit Alexa Fluor (1:200, Invitrogen, Cergy-Pontoise, France) antibodies. Nuclei were stained with Hoechst 33342 (1 μg/mL, Cell Signaling Technology). Cells in passages 3–5 were used for experiments.

2.5. Cell proliferation assay

The anti-proliferation effect of asiaticoside was investigated by the CCK-8 assay. PASMCs were cultured in 96-well plates in 200 μL of cell culture medium at a density of 3000 cells/well supplemented with 2% FBS (5% CO₂, 37 °C, 95% humidity) with asiaticoside (200 μg/mL, 100 μg/mL, 50 μg/mL, 25 μg/mL, 0 μg/mL; HA200, HA100, HA50, HA25, H) for 24 h in the hypoxic condition (3 wells in each group). PASMCs with 100 μg/mL asiaticoside (NA) or without asiaticoside (N) under normoxic conditions were used as controls. CCK-8 reagent (10 mL/well) was added to each well. The optical density (OD) value of each sample was tested at a wavelength of 450 nm on a microplate reader (ELX800, BioTek Instruments, USA), after incubation for 1 h at 37 °C. The results of cell viability measurements were expressed as the absorbance at OD450. Hypoxic cells were performed in a CO₂–N₂ incubator (Heraeus, Germany) at 5% O₂, 5% CO₂, and 90% N₂, 37 °C.

2.6. Cell migration assays

Cell migration was assessed by Transwell assays: cells were arrested by incubation in serum-free DMEM for 24 h. Then, PASMCs were divided into the following four groups: (1) normoxia + control (N), in which cells were cultured in serum-free DMEM with DMSO (0.05%) as control under normoxia (21% O₂, 5% CO₂); (2) normoxia + asiaticoside, in which cells were cultured in serum-free culture medium with DMSO (0.05%) and AS (100 μg/mL) for 48 h under normoxia conditions; (3) hypoxia + control, in which cells were cultured in serum-free DMEM with DMSO (0.05%) and AS (100 μg/mL) under hypoxic conditions (5% O₂, 5% CO₂); and (4) hypoxia + AS, in which cells were cultured in serum-free DMEM with DMSO (0.05%) and asiaticoside (100 μg/mL) under hypoxic conditions (3 wells in each group). In each group, approximately 100,000 cells were added to the top of a polycarbonate filter with 8-μm pores in basal medium. The cells were incubated for 6 h under control or hypoxic conditions. Cells remaining on the upper surface of the
filter were wiped off, and migrated cells were fixed in 90% ethanol for 15 min and stained with 0.1% crystal violet for 10 min. Therefore the quantity of crystal violet reflects the amount of the migrated cells. They were then washed twice and extracted by ethanoic acid for 15 min. The absorbance of leaching liquor, as an index of migrated cell number, was detected at 600 nm.

2.7. Detection of apoptosis

After treatment, the cells were fixed with 4% paraformaldehyde. Cells were subjected to a terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphatend nick end labeling (TUNEL) assay using the In-Situ Cell Death Detection Kit according to the manufacturer’s instructions. The cells for the TUNEL assay were grown on glass coverslips. TUNEL-positive cells were colored using diaminobenzidine (DAB) as the chromogen, and counterstained with hematoxylin. The percentage of TUNEL-positive cells was assessed in five randomly selected fields each section in a blinded fashion.

2.8. Caspase-3 activity assay

Caspase-3 activity was measured by a Caspase-3/CPP32 Colorimetric Assay Kit (BioVision, USA) based on spectrophotometric detection of the chromophore p-nitroaniline (pNA) after cleavage from the labeled substrate DEVD-pNA. Briefly, cells were lysed with chilled cell lysis buffer followed by centrifugation for 1 min at 10,000 g, and the supernatants (cytosolic extracts) were incubated with DEVD-pNA substrate at 37 °C for 2 h. The absorbance of pNA (400 nm) was measured for each sample on a microplate reader (ELX800, BioTek Instruments, USA). Results were calculated from a standard curve per the instructions of the manufacturer.

2.9. Real-time quantitative polymerase chain reaction

Quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed using the QuantiTect SYBR Green Kit to estimate the TGF-β receptor gene expression from rat lung tissue. Total RNA was extracted from lung tissue by Trizol, and RNA concentration was analyzed by a UV spectrophotometer to assess the quality of RNA. The concentration of extracted mRNA was determined using a real time RT-PCR assay with the Bio-Rad PCR 5100 system (Bio-Rad, Hercules, CA, USA). The specific primers of target genes and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed by Primer 5.0, as follows: TGF-βRI (forward: 5’GACAAGTCAGTTGCTCAG3’; reverse: 5’TCTCTTCAAATCTCTCAATG3’); TGF-βRII (forward: 5’ AAGATCCCTGACGGCTCTGA3’; reverse: 5’CTCTGCGTCTGGTCCATG3’); and GAPDH (forward: 5’CAACGGGAAACCCATCACCA3’; reverse: 5’ACGCCAGTAGAATACGACAT3’) (synthesized by Biolink).

Fig. 4. Effect of asiaticoside on proliferation and migration of PASMCs. (A) the upper image: Typical “hill and valley” appearance of rat PASMCs under phase contrast microscope; the under image: Immunofluorescent identification of α-smooth muscle actin was positive. (B) Demonstrative sections of rat PASMCs with crystal violet staining. (C) Cell migration by Transwell assay. (D) Cell number was tested with CCK-8. Compared with hypoxia + control group,* P < 0.05; compared with normoxia + control group,# P < 0.05, n = 3.
Fig. 5. Effect of asiaticoside on the expression of TGF-β1 in rat PASMCs. (A–B) Apoptosis was detected with a TUNEL assay. (C) Caspase-3 activity assay. (D) Densitometric analysis of TGF-β1 expression by Western blotting and demonstrative immunoblot of TGF-β1 in rat PASMCs. (E) TGF-β1 concentrations in the supernatant of rat PASMCs. Compared with normoxia + control group (N), *P < 0.05; compared with hypoxia + control group (H), †P < 0.05, n = 3.
Bio-technologies Co., Guangzhou, China). PCR detection threshold cycle (CT) values for each plate were calculated using the iCycler (Bio-Rad) software. For data analyses, the $2^{-\Delta\Delta Ct}$ values were determined.

2.10. Western blotting

The expression of TGF-β1, Smad2/3, and p-Smad2/3 in lungs of rats was detected by Western blot analysis. The TGF-β1 level in rats’ PASMCs was also measured after 24 h of coculture with drug treatments. Briefly, frozen lung tissue was prepared and homogenized in lysis buffer, sonicated twice and then centrifuged for 20 min at 3000 rpm. To determine the protein concentration of the lysate, the Bradford method was used with bovine serum albumin (BSA) as the standard. Equal amounts of proteins from each sample were resolved by SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, USA). Western blots were blocked by incubating with PBS containing 5% skimmed milk at 4 °C overnight. The membrane was then incubated with the primary antibodies [TGF-β1 (1:1000 dilution, Abcam), Smad2/3 (1:1000 dilution, Cell Signaling Technology), p-Smad2/3 (1:1000 dilution, Cell Signaling Technology) and anti-GAPDH (1:1000, Sigma)] overnight at 4 °C. Subsequently, the blots were incubated with the goat anti-rabbit secondary antibody (1:2000, Santa Cruz) for 1 h. By scanning the X-ray film, the optical density of the immunoblots was calculated with the Quantity one-4.6.2 software (Bio-Rad Laboratories, USA). All experiments were repeated at least three times.

2.11. Enzyme-linked immunosorbent assay (ELISA)

The levels of TGF-β1 in the supernatant of cultured rat PASMCs were measured by ELISA according to the manufacturer’s instructions (eBioscience). Data were quantified using a standard curve of known concentrations.

2.12. Statistical analysis

All statistical data were presented as mean ± standard deviation (SD). Between-group mean comparisons were performed using one-way analysis of variance (ANOVA) followed by a Student–Newman–Keuls test. P-values less than 0.05 were considered as statistically significant.

3. Results

3.1. Asiaticoside treatment prevents the development of HPH and right ventricle hypertrophy

As shown in Fig. 1A and D, in the hypoxia group, mPAP and RVI increased significantly compared with the normoxia + control group. Asiaticoside administered at 50 mg/kg daily for 4 weeks prevented the elevation of mPAP in chronic hypoxia exposed rats, while there was no significant difference between the AS group and control group in normoxic rats. Asiaticoside showed little repression of the systemic blood pressure, as carotid arterial pressure (CAP) which was increased by chronic hypoxia (Fig. 1B).

Four weeks after exposure to chronic hypoxia (9% O2), rats exhibited an increased ratio of the right ventricle to body weight (RV/body weight) and index of right ventricular hypertrophy (RVI: RV / LV + S) (Fig. 1C and D). The ratios of RV/body weight and RVI of rats in the hypoxia + AS group were lower than in the hypoxia + control group. These observations suggest that asiaticoside prevented cardiac hypertrophy. All differences showed statistical significance ($P < 0.05$ or $P < 0.01$).

3.2. Asiaticoside prevents the development of pulmonary vascular remodeling in HPH

Walls of pulmonary arterioles of rats in the normoxia + control group were thin and even. It was difficult to distinguish the intima, media, and extima (Fig. 2A and B). Compared with the normoxia + control group, the lumina of pulmonary arterioles in the hypoxia + control group decreased significantly, and inflammatory cells gathered along vascular walls. These abnormalities were greatly ameliorated by asiaticoside. Morphometric analysis of the degree of remodeling of pulmonary arterioles revealed that asiaticoside completely prevented the thickening of the media wall which was induced by hypoxia. Image analyses of pulmonary arterioles in each group are demonstrated in Fig. 2B.

3.3. Asiaticoside reduces the expression of TGF-β1, TGF-βRI and TGF-βRII and inhibits the phosphorylation of Smad2/3

Our data showed that the expressions of TGF-βRI and TGF-βRII were elevated in the lungs of hypoxia exposed rats while asiaticoside significantly inhibited TGF-β RI mRNA in hypoxia exposed rats (Fig. 3A and B). Asiaticoside seemed not to affect TGF-β RI mRNA (Fig. 3A). Also, the expression of TGF-β1 protein was increased in the lungs of hypoxia exposed rats. Asiaticoside prevented the rise in TGF-β1 in both normoxic and hypoxic rat lungs (Fig. 3C). Hypoxia exposure led to an increase in p-Smad2/3 activity, which was partly restored by asiaticoside treatment in both normoxic and hypoxic rats (Fig. 3E). The asiaticoside treatment did not significantly influence the expression of total Smad2/3 (Fig. 3D) despite the reduction of p-Smad2/3 levels, thereby indicating that the effects of AS on Smad2/3 are mainly at the phosphorylation level.

3.4. Asiaticoside inhibits migration and induces apoptosis of PASMCs in vitro

As shown in Fig. 4A, rat PASMCs were manifested using α-smooth muscle actin (α-SMA) as the marker by immunofluorescent staining. The migration of PASMCs was significantly inhibited by asiaticoside at concentrations of 100 μg/mL assessed by Transwell assays; however, 50 μg/mL asiaticoside had no significant inhibitory effect in normoxic PASMCs (Fig. 4B and C). To establish whether asiaticoside reduces cell numbers in isolated rat PASMCs, CCK-8 tests were carried out. The absorbance of PASMCs indicated the cell number. A significant decrease in absorbance was observed upon treatment with asiaticoside in a dose-dependent manner between 100 and 200 μg/mL (Fig. 4D). A TUNEL assay was then used to determine whether asiaticoside inhibited the cell number by inducing apoptosis in hypoxic PASMCs (Fig. 5A). As shown in Fig. 5B, the percentage of TUNEL-positive cells decreased in the H group, compared to the N group. And there were significant increases in TUNEL-positive cells in AS treated groups with AS concentrations from 25 μg/mL to 200 μg/mL after 24 h hypoxia exposure when compared with the H group. We also assessed the activity of Caspase-3 in these groups by the Caspase-3/CPP32 Colorimetric Assay Kit, which showed consistent results with the TUNEL test with AS concentrations of 50 μg/mL, 100 μg/mL and 200 μg/mL. Compared to the N group, Caspase-3 activity decreased in the H group. There were no significant differences between the H group and H25 group, although the latter shows a slight increase in Caspase-3 activity (Fig. 5C).

3.5. Asiaticoside inhibits the expression of TGF-β1 in rat PASMCs

Western blot analysis was used to measure the protein level of TGF-β1 in PASMCs. As shown in Fig. 5D, TGF-β1 had the highest expression levels under hypoxic conditions in rat PASMCs. Asiaticoside inhibited TGF-β1 expression in rat PASMCs in a dose-dependent manner except for asiaticoside at a concentration of 25 μg/mL under the hypoxic
condition (H25). Also, TGF-β1 expression in supernatant fluid was tested by the ELISA kit. The TGF-β1 concentrations in the supernatant of rat PASMCs increased in the hypoxia group (H), compared with the normoxia group (N). Asiaticoside at concentrations of 100 μg/mL (H100) and 200 μg/mL (H200) demonstrated a decreased expression of TGF-β1 in the supernatant of rat PASMCs, while there was no obvious inhibition observed in H25 and H50 (Fig. 5E).

4. Discussion

*C. asiatica* is a plant that has been used as a memory enhancer and psychotropic drug with a long history in traditional Ayurvedic medicine. In China, *C. asiatica* is widely used as a folk medicine in part because it is well tolerated. In this study we provide the first evidence that asiaticoside can prevent the development of HPH. Asiaticoside was given to hypoxia-exposed rats and resulted in prevention of the hypoxia-induced increases in mPAP and RVI, suggesting that asiaticoside can prevent the development of HPH in rats and attenuate right ventricle hypertrophy and suppressed hypoxia-induced vascular wall thickening.

Hypoxia plays a key role in the pathogenesis of HPH [31]. The pathological changes include pulmonary vessel constriction, increased proliferation and resistance to apoptosis of smooth muscle cells and structural remodeling of the heart and pulmonary arteries [18,26,35]. In line with a previous study, here we show that the development of hypoxia-induced pulmonary hypertension is associated with vascular remodeling, which is accompanied by proliferation and migration of PASMCs. In addition, we found elevated expression of TGF-β1 and TGF-β1 receptors in the lungs of rats, and our data revealed increased phosphorylation of Smad2/3 in rat lungs after hypoxia exposure. Chin et al. [8] comparatively studied the levels of TGF-β1 receptors in keloid fibroblasts and normal dermal fibroblasts, showing that there is an increased expression of TGF-β1 receptors I and II in keloid fibroblasts, which can be reduced by asiaticoside [33]. Our study showed that asiaticoside inhibited the mRNA expressions of TGF-βRII; however, asiaticoside did not alter TGF-βRII levels. The specific mechanism still needs to be studied further. Therefore, we determined that hypoxia elicited overactivity of TGF-β1 signaling, while asiaticoside markedly inhibited the increased expression of TGF-β1 and TGF-βRII and phosphorylation of Smad2/3. These data support the hypothesis that asiaticoside can regulate TGF-β1 signaling which is a pathogenic signaling pathway in HPH.

The aberrant proliferation and migration of PASMCs is a hallmark of the vascular remodeling in HPH. To offer more experimental groundwork of asiaticoside in the treatment of HPH, we investigated the effects of asiaticoside on PASMCs of rats in this study. We demonstrated that asiaticoside inhibits rat PASMC proliferation in a dose-dependent manner by inducing apoptosis of PASMCs in the hypoxia condition, which indicates a potential ability to be used as a recovery treatment in established HPH. The direct inhibition by asiaticoside of PASMCs might result in the suppression of vascular remodeling. Yet, it was also reported [19,20] that asiaticoside enhanced normal human skin cell growth, migration and attachment in vitro, and induced type I collagen synthesis via the activation of the TGF-β receptor I kinase (TβR I kinase)-independent Smad pathway, presenting a different side of asiaticoside. Diverse results might be explained by different cell types, experiment condition and duration of drug treatment. Therefore, the different roles of AS in different territories are worth further researching to get comprehensive recognition.

On the basis of our findings, we are convinced that asiaticoside can prevent HPH development in hypoxic rats. It is reasonable to speculate that early therapeutic intervention with asiaticoside in populations at high risk for HPH, especially those suffering from chronic hypoxia associated conditions such as obstructive pulmonary disease, bronchiectasia, altitude sickness and sleep related breathing disorders might provide favorable clinical outcome. Further research on whether asiaticoside can reverse established HPH needs to be undertaken. In addition, we chose the doses of asiaticoside used in the PASM C culture and in vivo rat model by reference to previously published literature. Further studies are required to determine the optimal dose and ideal course of treatment of asiaticoside.

5. Conclusions

In summary, we present novel data indicating that asiaticoside attenuates pulmonary hypertension, pulmonary vascular remodeling, and RV hypertrophy in HPH rats, which is likely mediated by blocking the hypoxia-induced overactivity of TGF-β1/Smad signaling and inducing apoptosis of the PASMCs. Given the preventative potential of the drug in the animal model and in vitro, we propose asiaticoside as a potential protective treatment in HPH.

Conflict of interest statement

The authors declare no conflict of interest.

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