

Pinoresinol diglucoside alleviates pressure overload-induced cardiac injury via the AMPK/SIRT3/RIG-1 pathway

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Abstract

Aims

Heart failure (HF) often results from mechanical or neurohormonal stress and remains the mortality up to 45–50%, which is accompanied by cardiomyocytes loss, apoptosis, and fibrosis. Pinoresinol diglucoside (PDG), a compound obtained from with *Forsythia suspensa*, *Epicommia ulmoides* and *Styrax sp.*, has a remarkable effect on anti-hypertension, anti-inflammation and anti-oxidative stress. However, whether PDG protects against pressure overload induced cardiomyocytes fibrosis, apoptosis and oxidative stress has not been illustrated. This study aimed to determine the cardioprotective effects and the underlying mechanism of PDG on cardiomyocytes injury in pressure overload-induced rats.

Methods

Abdominal aortic constriction (AAC)-surgery was performed to mimic pressure overload-induced cardiac injury (fibrosis, apoptosis, oxidative stress, and inflammation) in rats. The collagen content deposit evaluation of heart in rats were evaluated by PSR staining. Furthermore, phenylephrine (PE) was used to make in vitro cell models of cardiac injury. We used quantitative real-time PCR (qRT-PCR) to test the expression of targeting mRNAs both *in vivo* and *vitro*. Western blot assay was used to detect the protein expressions of the AMPK/SIRT3/RIG-1 signaling pathway.

Results

AAC induced the significant cardiac injury in hearts of rats, as indicators of myocytes fibrosis, apoptosis, oxidative stress and inflammation, whereas PDG treatment reversed these pathological changes compared to AAC rats. Moreover, AAC-induced increased left ventricular (LV) fibrosis, apoptosis, oxidative stress and inflammation compared to the Sham mice, but these increases were significantly reduced by PDG treatment. Interestingly, PDG in AAC-induced rats decreased the expressions of p-AMPK and SIRT3 at the protein levels. These results of this cardio-protection are likely through targeting cardiomyocytes fibrosis, apoptosis, oxidative stress and inflammation, possibly mediated by AMPK and SIRT3. In addition, PDG also blocked PE-induced the fibrotic changes in cardiomyocytes. Thus, PDG may be a potential therapeutic agent in targeting pathological cardiac injury by inhibiting myocytes fibrosis, apoptosis, oxidative stress and inflammation in pressure overload-induced cardiac injury.

Conclusions

PDG significantly decreased AAC-induced cardiomyocytes fibrosis, apoptosis, oxidative stress and inflammation in rats through the AMPK/SIRT3/RIG-1 signaling pathway. These novel findings provide the evidence that PDG may be a promising therapeutic strategy for pathological cardiac remodeling and HF.

Introduction

In the past 20 years, there is almost no change in the mortality of heart failure (HF), and the 5-year mortality of HF is up to 45–50% [1]. It is well-established that HF is accompanied by cardiomyocyte loss, apoptosis and oxidative stress [2], upregulation of cardiac dysfunction, and cardiomyocytes fibrosis [3, 4]. Currently, some chemical synthetic drugs such as angiotensin-converting enzyme inhibitors, β -adrenergic receptor agonists, angiotensin receptor blockers, and aldosterone antagonists are primarily used to treat HF [5]. However, limited data on the long-term beneficial actions of these chemical drugs in treating HF has been reported, and often accompanied by some side effects. Recently, a large of natural drugs are well-recognized to be protective against cardiac remodeling in HF. Thus, we speculate that natural bioactive compounds may provide a promising treatment option for treating HF.

Pinoresinol diglucoside (PDG) is obtained from *Eucommia ulmoides*, *Forsythia suspensa*, as well as *Styrax sp.* [6–13]. PDG alleviates the inflammation induced by lipopolysaccharide (LPS) [14]. Moreover, PDG significantly decreases oxLDL-induced up-regulation of LOX-1 and ICAM-1, lipid peroxidation and production of ROS in endothelial cells [15]. Recently, our findings suggest that PDG has a cardioprotective role against myocardial hypertrophy and inflammation induced by pressure overload in rats [16]. These results indicate that PDG has cardioprotective effects against cardiac hypertrophy and inflammation both *in vivo* and *in vitro*, as well as oxidative stress in endothelial cells. However, whether PDG has a potential role in the prevention of cardiac fibrosis, apoptosis, and oxidative stress in pressure overload-induced rats has not been illustrated.

Adenosine monophosphate-activated protein kinase (AMPK), an established energy sensor in the heart, which can protect the heart against heart hypertrophy and HF [17]. Inhibiting AMPK directly promotes cardiac hypertrophy and the progression of HF [18, 19]. SIRT3, a mitochondrial enzyme involved in energy homeostasis in various cardiovascular disorders, can also protect the heart against oxidative stress [20]. Retinoic acid-inducible gene 1 (RIG-1) is one of the important pathogen recognition receptors (PRRs) [21], which can activate important transcription factors that facilitate reprogramming. As such, we speculate that in the fight against cardiac hypertrophy and HF, therapeutic modulation of AMPK/SIRT3/RIG-1 signaling may be useful, but this goal remains unachievable without effective pharmacological treatments.

This research aimed to investigate whether PDG treatment protects the heart against pressure overload-induced cardiac injury. Here, we reported that a significant reduction in fibrosis, apoptosis, oxidative stress, and inflammation when observed after PDG administration to the heart may be through the AMPK/SIRT3/RIG-1 signaling pathway.

Materials And Methods

Chemicals and agents

Fetal bovine serum (FBS) was obtained from Bovogen (New Zealand). Phenylephrine (PE), Dulbecco's modified eagle's medium (DMEM) cocktail were obtained from Corning (Jiangsu, China). A mixture of penicillin streptomycin was purchased from Wuhan Servicebio Technology. The BCA Protein Reagent kit was purchased from Thermo. The primary antibodies used were as followed: α -SMA (D4K9N, Cell Signaling Technology), AMPK (#5831, CST), *p*-AMPK (#2535, CST), SIRT3 (#5490, CST), RIG-1 (#3743, CST), GAPDH (TA-08, ZSGB-BIO). The goat anti-rabbit IgG-HRP (SA00001-2, Protech) and goat anti-mouse IgG-HRP (SA00001-1, Protech) were used according to the protocol of the manufacturer. The primers of GAPDH, TNF- α , IL-1 β , IL-6, collagen I (Col I), collagen III (Col III), Periostin and α -SMA were synthesized by Sangon-Bio (Shanghai, China). MDA, SOD and GSH-Px kits were supplied by Changzhou Redox Biological Technology Corporation (Jiangsu, CN).

Preparation of PDG

PDG (MW = 682.668 kDa) was obtained from Oliver bark of *Eucommia ulmoides*, which was purchased from Manster Biotechnology Co. (Chengdu, China). The purity of PDG (CAS, A0268) is no less than 99%. PDG solution in PBS was prepared at 2 mg/ml and stored at 4°C within 24 h.

Animal experiments

Animals were housed on a 12 h light-dark cycle with access to free water and food, at the temperature from 18°C to 26°C and humidity from 40% and 70% in Weifang Medical University Experimental Animals Center. 8-week-old male SD rats, purchased from Shandong Pengyue Experimental Animal Center (Jinan, China) with the weight of 180 ± 20 g, were used to generate an in vivo models of chronic cardiac injury induced by AAC-surgery.

Here, abdominal aortic constriction (AAC) surgery was used to mimic pressure overload to produce an *in vivo* models of chronic cardiac injury, as an indicator of cardiomyocytes fibrosis, apoptosis, oxidative stress and inflammation in rats. AAC-surgery was operated on as previously described [22]. In brief, making a 3 cm-incision along the abdomen midline and exposing the abdominal aorta at a site above the left renal artery, it was to insert a 21 G syringe needle parallel to the aortic artery with a 4 – 0 silk suture loop to a tightened knot around the aorta, ultimately, the needle was removed. The Sham surgery was as the same as the AAC procedure except with no ligation above the left renal artery. All rats were administered with penicillin for 7 days to prevent the infection resulting from AAC-surgery.

After AAC-surgery for 7 days, the survived rats were randomly assigned into six groups (n = 6/group): (1) Sham group + vehicle-treatment: rats were performed the sham surgery and treated vehicle by intraperitoneal injection (i.p.); (2) AAC group + vehicle-treatment: rats were performed AAC surgery and treated with vehicle by i.p. (3) AAC group + L-PDG: rats were performed with AAC-surgery and given low-dosage PDG (L-PDG, 2.5 mg/kg/d) by i.p. (4) AAC group + M-PDG: rats were performed with AAC-surgery and given medium-dosage PDG (M-PDG, 5 mg/kg/d) by i.p. (5) AAC group + H-PDG: rats were performed with AAC-surgery and given high-dosage PDG (H-PDG, 7.5 mg/kg/d) by i.p. (6) Sham control + H-PDG: rats were performed with the Sham surgery and treated with high-dosage PDG (H-PDG, 7.5 mg/kg/d).

The experimental protocols were shown in Fig. 1A. After treated with PDG for 3 weeks post AAC-surgery 4 weeks, rats were euthanatized with 20% urethane, and LV tissues were harvested to evaluate the histological changes and target mRNAs and protein expression in the hearts of rats.

Pathological changes of heart in AAC-induced rats

With 4% paraformaldehyde, LV samples were fixed within 24 h, then embeded in by paraformaldehyde, and finally triformol-fixed. PSR staining for histological changes assessments were evaluated. Typical images were photographed and analyzed under an optic microscope.

Isolation of primary neonatal ventricle myocytes and fibroblasts (NRVMs and NRVFs)

To isolate NRVMs within 48 h, 0.1% trypsin and 0.03% collagenase were used to dissolve them by enzymatic dissociation, as described previously [23]. SD rats between 1-day to 2-day were supplied by Shandong Pengyue Experimental Animal Center (Jinan, China) and isolated NRVMs and NRVFs for *in vitro* cell experiments. As required by the experiments, 3×10^6 /ml cells were cultured for 24 hours and then used for Western blot analysis and quantitative real-time PCR analysis(qRT-PCR).

NRVFs were randomly assigned into six groups: (1) Control groups were added with vehicle. (2) Control group + H-PDG: cells were given PDG (H-PDG, 7.5 ug/ml). (3) PE group: cells were given PE-stimulation (50 μ M) for 48h. (4) PE group + L-PDG: cells were given PE and given PDG treatment (L-PDG, 2.5 ug/ml). (5) PE group + M-PDG: cells were given PE and PDG treatment (M-PDG, 5 ug/ml). (6) PE group + H-PDG: cells were given PE and PDG treatment (H-PDG, 7.5 ug/ml).

qRT-PCR

Total RNA was extracted from total heart tissue and synthesized to cDNA with a reverse transcription Kit (TOYOBO, Japan). cDNA was real-time quantified by gene primers and SYBR Green Master Mix (TOYOBO, Japan), conducted on the Cycler 480 System (Roche, German). The expression of all target genes was normalized to that of the reference gene GAPDH. The primers we used are as follows (Table 1). The relative expression was analyzed by the $2^{-\Delta\Delta CT}$ method.

Table 1
Primers for the real-time polymerase chain reaction.

Gene	Forward (5'to 3')	Reverse (5'to 3')
GAPDH	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT
α -SMA	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA
Periostin	TGGTGTGTCCATGTCATCGA	TGTGAAGTGACCGTCTCTTCCA
Collagen I	GGAACCTTCGCTTCCATACTC	ACTGGTACATCAGCCCAAAC
Collagen III	GCCATCCTCTAGAACTGTGTAAG	CAGGCCAATGGCAATGTAAAG
TNF- α	GCATGATCCGAGATGTGGAAGTGG	CGCCACGAGCAGGAATGAGAAG
IL-1 β	ATCTCACAGCAGCATCTCGACAAG	CACACTAGCAGGTCGTCATCATCC
IL-6	AGGAGTGGCTAAGGACCAAGACC	TGCCGAGTAGACCTCATAGTGACC

Western blot

Extract the heart tissue with RIPA buffer. Softly stirred on ice for 40 min and centrifugated for 20 min at 4 °C, the supernatants were harvested. The protein concentration was detected by the BCA Protein Detection Kit (Thermo Scientific). The primary antibodies are used as the following: anti-AMPK (1:1000), P-AMPK (1:1000), SIRT3 (1:1000), RIG-1 (1:1000). The ratio of secondary epitaplate antibodies is 1:2000. Protein bands were evaluated and analyzed using the Chemiluminescence Fluor Chem FC3 system (Protein Simple, USA).

Enzyme-linked immunosorbent assay (ELISA)

Serum samples were collected and analyzed by ELISA using SOD, GSH-PX, MDA ELISA kits (Jiangsu, CN) in accordance with the manufacturer's instructions. ELISA readers were used to measure the optical densities at 560nm, 412nm, and 450nm for each well. A standard curve prepared with standard solutions at different concentrations within the kit was used to calculate the quantity of each sample.

TUNNEL staining

Assays for apoptosis are performed using TdT-mediated dUTP Nick-End Labeling (TUNEL). Briefly, after de-waxing and rehydrating the tissue sections, proteinase K was used as a permeabilization agent and then with TUNEL reagent (Vazyme Biotech Co.,Ltd). We stained the nuclei with 4, 6-diamidino-2-phenylindole (DAPI), illuminating positive nuclei in green, and DAPI stained nuclei in blue. We obtained photomicrographs using fluorescent microscopes.

Statistical analysis

Data were presented as means $\pm$ standard deviation (SD). Multiple comparisons among three or more groups were analyzed by one-way ANOVA followed by an LSD-test, P 0.05 was considered as a

significant difference.

Results

Effect of PDG on cardiac fibrosis induced by abdominal aortic constriction (AAC) surgery in rats.

To investigate the therapeutic effect of PDG on the heart under pathological conditions, we used abdominal aortic constriction (AAC) surgery in rats to create an *in vivo* model to mimic pressure overload according to the previously reported [22]. The experimental protocols were shown in Fig. 1A. Previous evidence showed that excessive collagen deposits are closely linked to myocardial fibrosis [24]. Herein, AAC surgery induced considerably more perivascular and interstitial fibrosis in rats, while PDG treatment significantly decreased the abnormal deposition of collagen fibers in AAC rats (Fig. 1B-C). In addition, AAC surgery also significantly increased the expressions of Col I and Col III at the protein levels, while PDG administration reversed these fibrotic changes in AAC rats (Fig. 1D-E). Consistently, Periostin [25] and α -SMA [26], the markers of the non-cardiomyocyte lineage and transformation from fibroblast to myofibroblasts, as well as showed an upward trend after AAC operation, but were significantly decreased after PDG administration (Fig. 1F-G). The results of cardiac fibrosis induced by AAC surgery are similar to the previous study [27], and the therapeutic effect of PDG suggests that it could alleviate cardiac injury by suppressing cardiac fibrosis.

Effect of PDG on cardiomyocyte apoptosis in AAC-induced rats.

The process of programmed cell death, apoptosis, is an adaptive response, whereas extensive apoptosis can lead to cardiac dysfunction [28]. To further examine the effect of PDG on pressure overload, we analyzed the cell apoptosis in heart tissues treated with PDG after AAC surgery. In the heart tissue from the sham surgery, there were < 20% of cells that stained positively with TUNEL, indicating that the tissue was in a relatively normal physiological state. On the contrary, AAC surgery caused widespread TUNEL-positive nuclei in tissues, but they were clearly reduced by PDG treatment (Fig. 2A-B). TUNNEL staining showed that AAC surgery increased heart cell apoptosis, but PDG significantly alleviates cell apoptosis, suggesting PDG has a potential therapeutic effect on heart injury.

Effect of PDG on oxidative stress and inflammation in AAC-induced rats.

A proliferation of reactive oxygen species and a deficiency of antioxidant defense mechanisms lead to oxidative stress, which is harmful to antioxidant defense mechanisms (SOD and GSH-Px) [29] or increased malondialdehyde (MDA) levels [30], thereby leading to an increase in ROS levels. Using ELISA assay, we found that the protein expression of SOD significantly decreased in AAC rats, but GSH-Px and MDA levels increased, while PDG treatment could reverse these changes (Fig. 3A-C). On the other hand, we verified that PDG administration can reduce the mRNA level of inflammatory cytokines in the myocardium in AAC rats (Fig. 3D-F), indicating that PDG can improve the inflammatory response induced by pressure overload, in line with the previous study [16].

Effect of PDG on fibrotic biomarkers in PE-induced NRVFs.

To confirm the direct effect of PDG on cardiac fibrosis, we isolated and cultured NRVFs from SD rats within 48h. *In vitro*, cell models of pressure overload were established by inducing NRVFs with PE (50 μ M) for 48h. Consistent with the previous studies, the relative mRNA levels of *Col I* and *Col III* [31] were increased after PE treatment, as well as the periostin [32] and α -SMA [33] expression. The mRNA expression levels of these fibrotic biomarkers were significantly reduced by high dosage of PDG treatment (7.5 μ g/ml) (Fig. 4A-D). Similarly, Western blot analysis revealed that the α -SMA level in NRVFs treated with PE was significantly increased compared with the control group, while the PDG treatment decreased the level of α -SMA compared with the PE group. With a high dosage of PDG treatment in the absence of hypertrophic stimuli, the level of α -SMA was comparable to that of the control group (Fig. 4F-G). These data suggest that PDG has a protective effect on cardiac fibrosis.

PDG blunts cardiac injury through the AMPK/SIRT3/RIG-1 signaling pathway *in vivo*.

It has been widely confirmed that decreasing AMPK/SIRT3 pathway levels can cause cardiac injury [34]. Therefore, we first verified the effect of PDG on AMPK/SIRT3 pathway. Western blotting analysis showed that p-AMPK and SIRT3 protein expressions significantly decreased in AAC rats, while total-AMPK expression changed slightly or remained unchanged with no statistical significance. PDG treatment significantly alleviated the inhibition of the AMPK/SIRT3 pathway by pressure overload but had no significant effect on the sham group (Fig. 5A-C). Additionally, retinoic acid inducible gene 1 (Rig-1) has been confirmed to mediate impaired cell death and immune responses [35–37]. Our results showed that compared to the sham group, the AAC group showed a significant reduction in RIG-1 expression. However, the expression level of the above protein factor was significantly increased in the myocardial tissue of the mice after PDG treatment as compared with the AAC group (Fig. 5A-D). The reversal effect of PDG on the expression level of RIG-1 under pressure overload suggests that PDG plays a significant role in the treatment of myocardial injury. Collectively, these data suggest that PDG treatment effectively activates the AMPK/SIRT3/RIG-1 signaling pathway *in vivo*.

Discussion

PDG, purified from *Eucommia ulmoides*, *Forsythia suspensa*, and *Styrax sp.*, is well-identified to have effects against hypertension [7, 8], inflammation [14], oxidative stress [15], osteoporosis [9] and other pharmacological activities. Recently, many strong evidences showed that PDG could reduce neuroinflammation, apoptosis, as well as oxidative stress [38, 39]. In addition, PDG acts directly on smooth muscle cells in blood vessels to dilate in PE - induced vascular models [40], and human umbilical vein endothelial cells benefit from PDG by alleviating oxLDL-induced dysfunction. [15]. PDG could prevent AAC-induced cardiac hypertrophy *in vivo*, PE- and ISO-induced cardiac hypertrophy *in vitro* [16]. Herein, we found that cardiomyocyte fibrosis, apoptosis, oxidative stress, and inflammation induced by AAC can be effectively alleviated and reversed by PDG treatment in rats (Figs. 1–4). Further studies showed that PDG

could play a protective role in the myocardial remodeling model in mice by activating the AMPK/SIRT3/RIG-1 signaling pathway *in vivo* (Fig. 5).

It is well-recognized that the development of HF is associated with left ventricular dysfunction caused by myocardial interstitial fibrosis [41]. Fibrotic biomarkers of *α-SMA*, *periostin*, *Col I*, and *Col III* play a strong part, which are often determined as a clinical index of cardiac fibrotic injury [42–44]. Here, our results showed for the first time that PDG administration alleviated AAC-induced fibrotic changes of heart in rats. mRNA expression levels of related fibrosis indicators increased in both AAC-induced rats and PE-stimulated NRVFs, but the indexes of each group recovered after PDG treatment (Figs. 1 and 4). These data suggest that AAC-surgery can accelerate myocardial fibrosis, while PDG can alleviate and reverse pathological changes to achieve myocardial protection.

Cardiomyocyte loss, which can be extensively described to occur through necrosis, apoptosis, or autophagy [45] can exacerbate cardiac dysfunction. Apoptosis is defined as a highly choreographed form of cell death that occurs in failing hearts [46]. Sustained extensive apoptosis leads to loss of cardiomyocytes, leading to fatal heart failure [47]. Some previous studies have suggested that apoptosis increases the risk of heart failure in diabetics [48, 49]. While an important factor in apoptosis is oxidative stress, which is characterized by low antioxidant levels (such as SOD) and high production of oxidative intermediates (such as ROS). [50]. Extensive studies confirmed that oxidative stress and inflammation are both enhanced in the development of chronic heart failure [51–53]. Mitochondrial dysfunction causes oxidative stress, which in turn destroys cellular components contributing to heart failure by damaging and inflaming myocardial tissue [54]. By ELISA assay and TUNEL assay, our results are consistent with the above conclusions. However, PDG treatment significantly cured and reversed these pathological changes. Thus, our findings indicate that PDG inhibited AAC-induced oxidative stress, inflammation, and apoptosis *in vivo*, and PDG was dose-dependent in the treatment of cardiac injury at the same time (Figs. 2–3).

It has been reported that heart stress is orchestrated by SIRT3-AMPK, a crucial transcriptional regulator of cardiac metabolism [55]. The role of this pathway in regulating a wide range of key pathological processes, including fibrosis, apoptosis, oxidative stress, and inflammation, has been well studied [56–58]. Meanwhile, RIG-1 pathway has been proved to have an effect on regulating immunity, oxidative stress and anti-inflammation [59, 60]. Therefore, supported by the evidence that PDG inhibits the inflammatory response and improves myocardial hypertrophy in rats [16], our findings showed that the inhibitory effect of PDG on pathological changes such as myocardial fibrosis and apoptosis might be related to the AMPK/SIRT3/RIG-1 pathway (Fig. 5).

In this study, we first reported that the administration of PDG could ameliorate pressure overload-induced cardiac injury both *in vivo* and *in vitro*. There is no denying that not only the expressions of fibrotic biomarkers of *α-SMA*, *periostin*, *Col I* and *Col III*, pro-inflammatory cytokines TNF-α, IL-1β and IL-6, but also the levels of oxidative stress and apoptosis all significantly reduced after 3-week administration of PDG. Furthermore, our data first confirmed that AAC surgery limits the expression of the AMPK/SIRT3/RIG-1

signaling pathway; however, PDG treatment significantly reversed these changes. However, the *in vitro* experiment of this study only verified the changes of fibrosis indicators, which was a limitation of this study. In addition, the optimal dose cannot be obtained according to the therapeutic effect of different doses of PDG, thus the pharmacokinetics of PDG deserves attention. Generally speaking, the study reveals that the important role that PDG administration may play in ameliorating the development of cardiac injury and suggests that PDG may be a new drug candidate for the treatment of HF. This beneficial effect of PDG may be related to the inhibition of the AMPK/SIRT3/RIG-1 signaling pathway.

Conclusions

Our findings suggest that PDG has a promising therapeutic effect on cardiac injury in pressure overload by reducing myocyte fibrosis, apoptosis, inflammation, and oxidative stress through the AMPK/SIRT3/RIG-1 pathway.

Declarations

Abbreviations

PDG, Pinorensinol diglucoside; SD, Sprague Dawley; NRVMs, neonatal rat ventricular cardiomyocytes; HF, heart failure; PE, phenylephrine; TNF- α , tumor necrosis factor- α ; AAC, abdominal aortic constriction; IL-6, interleukin-6; IL-1 β , interleukin-1 β .

Ethics approval and consent to participate

Animal experiments were carried out according to the Guide for the Care and Use of Laboratory Animals issued by the National Institutes of Health (NIH) and Shandong Municipal People's Government. All animal experiment protocols were approved by the Animal Care and Use Committee of Weifang Medical University (2021SDL019).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

LZ designed and supervised the experiments; YG, WJ, and TL conducted the experiments; QY and SF performed the isolation of NRVMs and PDG treatment; DX, FW and XY established the *in vivo* model in rats; LZ wrote the manuscript.

Data Availability Statement

All supporting data within the article are available upon reasonable request from any qualified investigator.

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Not applicable.

Consent for publication

Not applicable.

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Figures

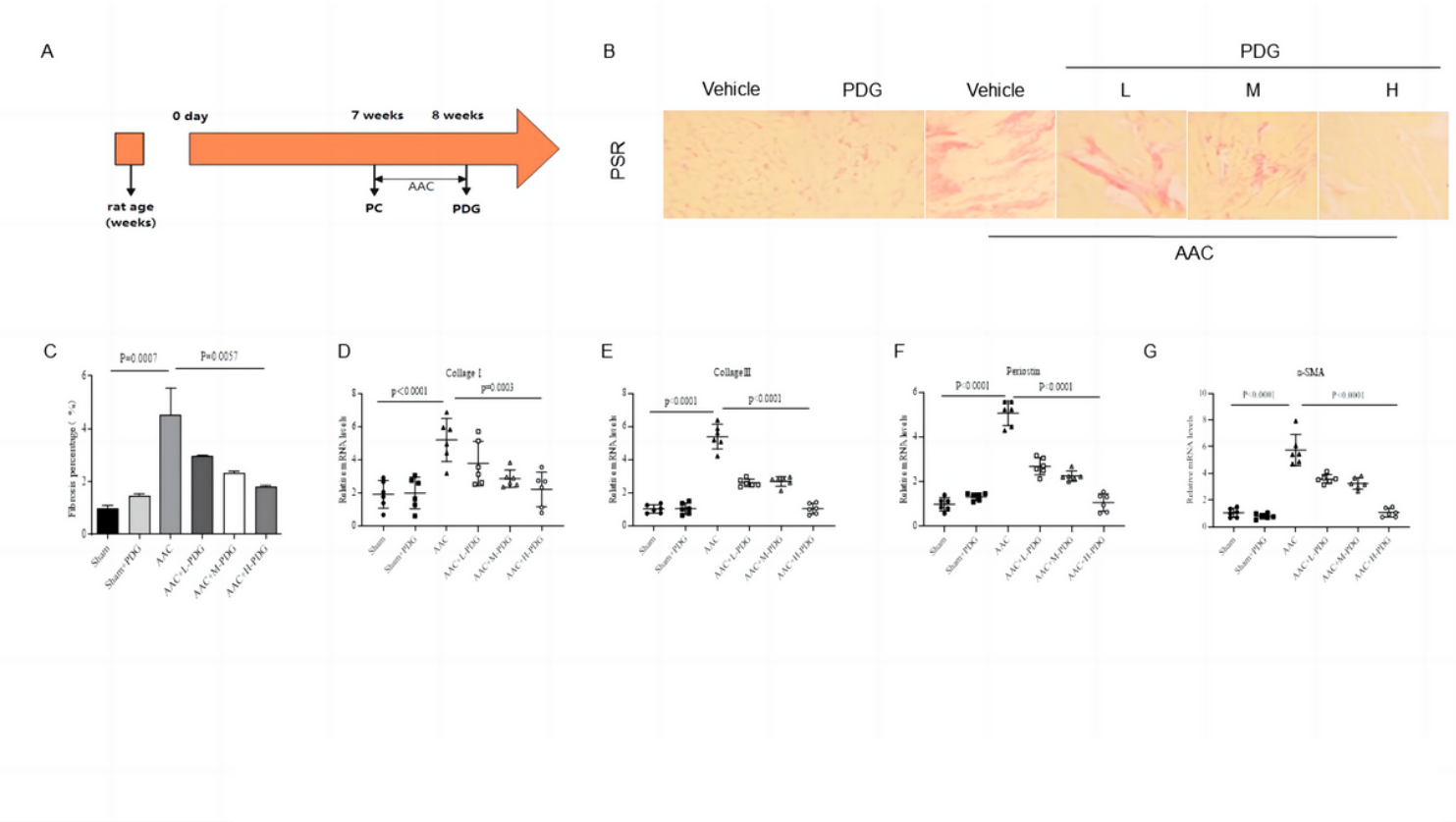


Figure 1

Effect of PDG on cardiac fibrosis in hearts of AAC-induced rats.

The pressure overload in vitro model was completed over four weeks. Different doses of PDG were injected into rats in three treatment groups, and other control groups were injected with equal amounts of normal saline (NS) or PDG. (A) displays the experimental design protocol. Low dose PDG (L-PDG, 2.5 mg•kg⁻¹•d⁻¹), medium dose PDG (M-PDG, 5 mg•kg⁻¹•d⁻¹) and high dose PDG (H-PDG, 7.5 mg•kg⁻¹•d⁻¹) was administered by i.p. one week after AAC surgery. AAC surgery was not performed in Sham group. (B) Representative images of PSR staining of the myocardium in 6 groups of rats (Bar = 50 μm). (C) Quantification of fibrosis percentage () in 6 groups of rats. (D-G) Relative mRNA expression of fibrosis markers Collage , Collage , Periostin and α-SMA in the heart tissues of the six groups. Results were presented as mean ± SD. n=6 per group. The P values are shown in the figure.

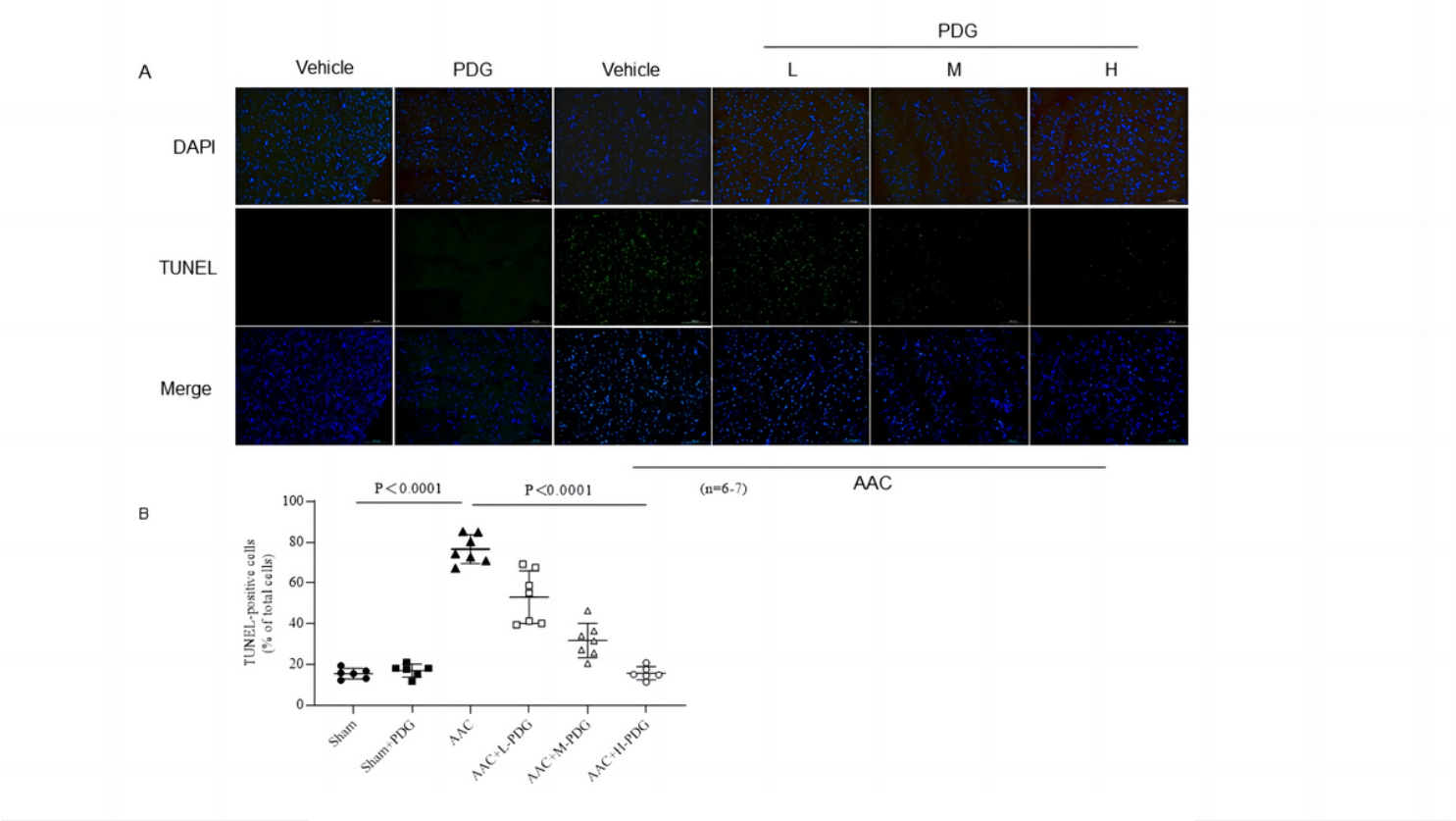


Figure 2

Effect of PDG on cardiomyocytes apoptosis in AAC-induced rats.

(A) Representative photographs of TUNEL staining of 6 groups of rats in heart sections (Bar = 100 μm). (B) Quantitative analysis of TUNEL staining of 6 groups of rats in heart sections. n=6-7 per group. The data are expressed as mean ± SD. P < 0.0001 versus vehicle-Sham group; *p* < 0.0001 versus the AAC group.

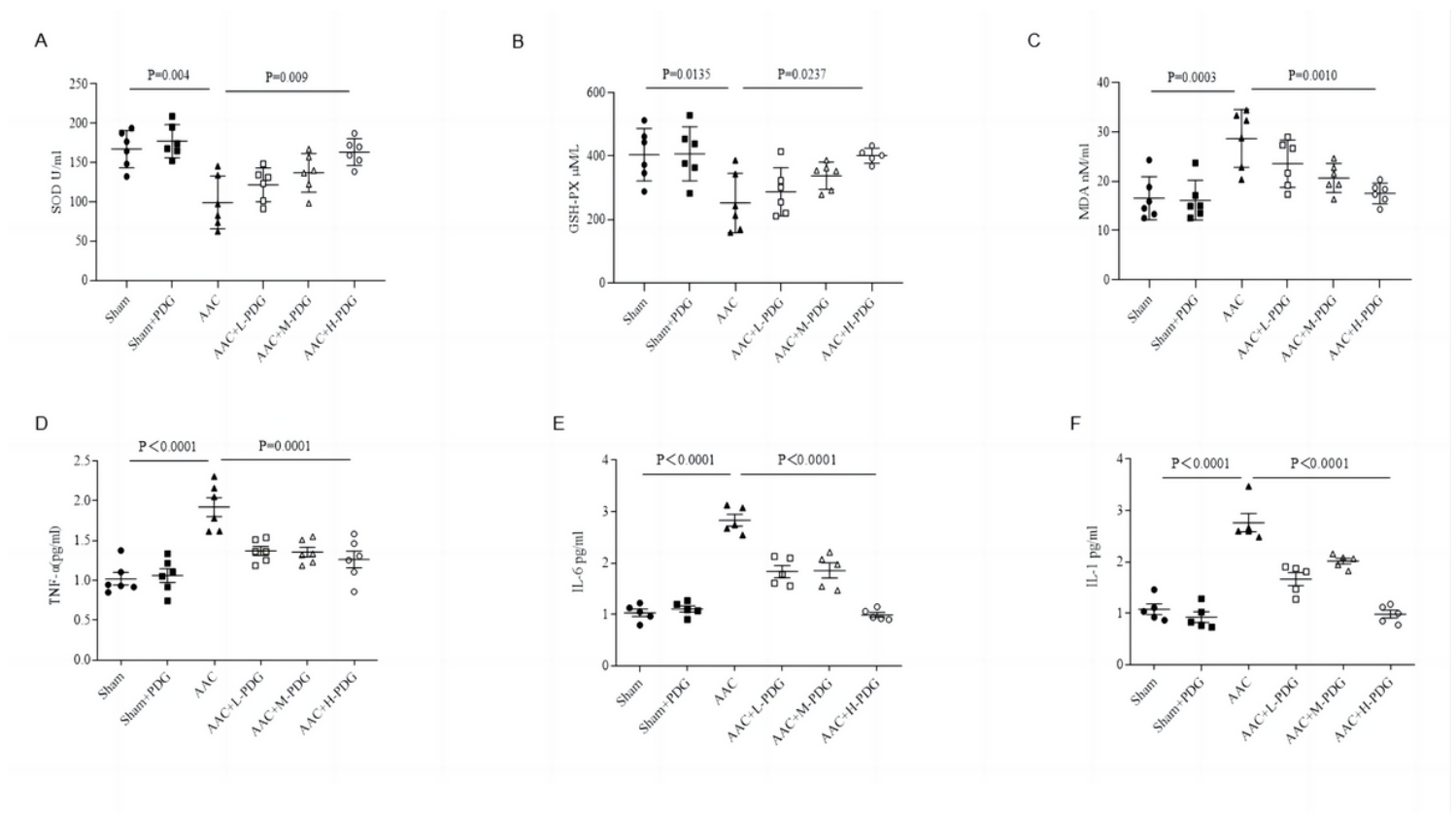


Figure 3

Effect of PDG on oxidative stress and inflammation in AAC-induced rats.

(A) Representative superoxide dismutase (SOD) levels in serum of six groups of rats. (B) Representative glutathione peroxidase (GSH-PX) levels in serum of six groups of rats. (C) Representative malondialdehyde (MDA) levels in serum of six groups of rats. (D) Representative TNF- α mRNA expression in the heart tissues. (E) Representative IL-6 mRNA expression in the heart tissues. (F) Representative IL-1 β mRNA expression in the heart tissues. Three inflammatory indicators are normalized to GAPDH. n=6 per group. The data are expressed as mean $\pm$ SD. The P values are shown in the figure.

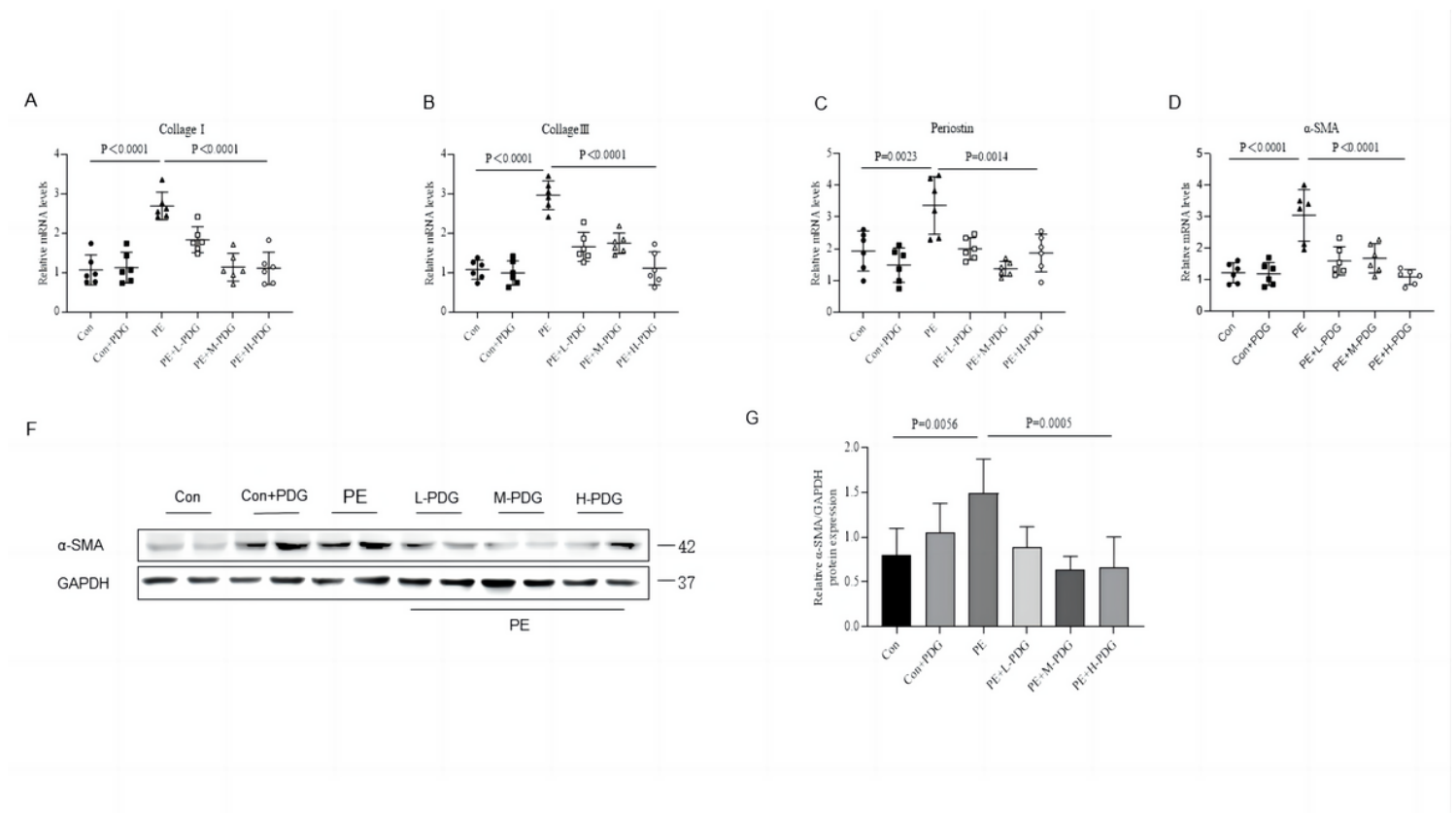


Figure 4

Effect of PDG on fibrotic biomarkers in PE-induced NRVFs.

(A-D) Relative mRNA expression of fibrotic markers Collage , Collage , Periostin and α-SMA in 6 groups of NRVMs. (E) Western-blot analysis of α-SMA protein expression in 6 groups of NRVMs. (F) Quantification of α-SMA expression in 6 groups of NRVMs. GAPDH was used as an internal control. The data are showed as mean ± SD. n=6 per group. The P values are shown in the figure.

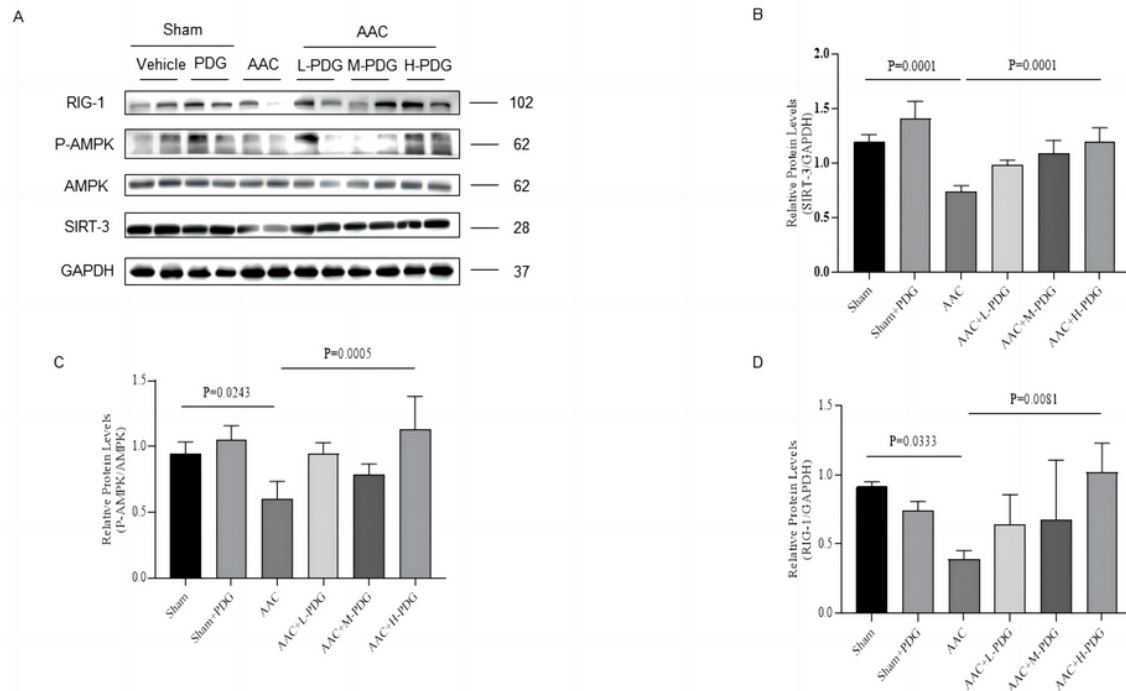


Figure 5

PDG blunts cardiac injury through the AMPK/SIRT3/RIG-1 signaling pathway.

(A) Immunoblotting analyses of *p*-AMPK, AMPK, SIRT3 and RIG-1 protein expression. (B-D) Quantification of *p*-AMPK, AMPK, SIRT3 and RIG-1 expressions in six groups of rat heart tissue. GAPDH was used as an internal control. The data are expressed as mean $\pm$ SD. n=6 per group. The P values are shown in the figure.

Supplementary Files

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