JAK2/STAT3 pathway mediates beneficial effects of pterostilbene on cardiac contractile and electrical function in the setting of myocardial reperfusion injury

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Short title: Cardioprotective effects of pterostilbene in reperfusion injury

Abstract

Background: Contractile dysfunction and fatal arrhythmias are the hallmarks of myocardial ischemia/reperfusion (I/R) injury. Pterostilbene has notable cardioprotective effects, but its main mechanisms are not fully understood. Here, we investigated the effect of PTE on myocardial hemodynamics, arrhythmias, inflammatory/oxidative responses, and the causal role of the JAK2/STAT3 pathway in rats with acute myocardial I/R injury.

Methods: Sixty male 7-8 months Sprague-Dawley rats (n=10/each group) experienced *in vivo* model of myocardial I/R injury through 40-min LAD coronary artery occlusion and subsequent 24-h reperfusion. PTE at concentrations of 5 and 25 mg/kg was intraperitoneally administered to rats five min before reperfusion. Cardiac hemodynamics, reperfusion-induced ventricular arrhythmias, infarct size, inflammatory cytokines, oxidative stress markers, the activity of the JAK2/STAT3 pathway were measured as the endpoints.

Results: Administration of PTE to I/R-injured rats recovered myocardial contractile function and reduced infarct size and ventricular arrhythmias counts and incidence in a dose-dependent manner. PTE at 25 mg/kg significantly and more potently reduced the levels of inflammatory mediators NF-κB, TNF-α, and IL-1β, suppressed intracellular ROS production, augmented the activity of glutathione, and manganese-superoxide dismutase, and upregulated the JAK2 and STAT3 phosphorylation. Importantly, pretreatment of rats with Ag490 as a JAK2 inhibitor significantly abolished the cardioprotective and signaling effects of PTE in I/R rats.

Conclusion: PTE exerts significant protective effects on reducing arrhythmias and myocardial infarction and enhancing cardiac function by stimulating JAK2/STAT3-related suppression of inflammatory and oxidative reactions in the I/R injury setting.

Keywords: Arrhythmia; Ischemia; Reperfusion; Signaling pathway; Pterostilbene; JAK2/STAT3

Introduction

Myocardial ischemia is a major feature of coronary heart disease, which occurs due to sudden or gradual blockage of the coronary arteries and microcirculatory disorders [1]. Restoration of blood flow to the ischemic region, although necessary, causes ischemic ischemia/reperfusion (I/R) injury and imposes functional and structural damages to the heart. Reperfusion-induced arrhythmias are also dominant clinical manifestations that limit reperfusion therapy in the clinic [2]. The pathophysiology of myocardial I/R injury is numerous and includes endothelial and microvascular disorders, myocytes apoptosis, necrosis and swelling, destructive inflammatory responses, and oxidative stress, which together expand the infarct size and reduce the beneficial effects of reperfusion [3,4]. Therefore, in order to reduce the fatal side effects of reperfusion therapy, introducing powerful treatments is of clinical importance.

During periods of ischemia and reperfusion, the production of reactive oxygen species (ROS) from various sources increases, irritating oxidative stress. Numerous studies have shown that oxidative stress is associated with cardiac cell death in ischemic lesions [4,5]. In addition, increased ROS causes cytochrome C to leak from the mitochondria and worsens cardiomyocyte death after I/R injury by recruiting caspases. Following these events and simultaneously with them, an inflammatory reaction also occurs and exacerbates heart dysfunction [5]. The inflammatory response plays a key role in the occurrence of secondary heart damage. Inflammatory mediators including nuclear factor kappa B (NF-κB) and pro-inflammatory cytokines have an important contribution in the development of infarct size and incidence of lethal arrhythmias in reperfusion [6,7].

There is ample evidence that the Janus kinase-2/transducer signal transducer and transcript pathway-3 activator (JAK2/STAT3) plays crucial roles in mediating the cardioprotective effects

of pre- and post-conditioning therapeutic interventions against myocardial I/R injury [8]. Activation of JAK2 induces STAT phosphorylation, which in turn binds to specific DNA elements and promotes transcription of antioxidant and anti-inflammatory genes. This pathway belongs to the main survivor activating factor enhancement pathway (SAFE) whose activity is significantly reduced in I/R insults [9,10]. Protective conditioning interventions reduce I/R outcomes by improving cardiac hemodynamics, reducing the size of myocardial infarction, and inhibiting cardiomyocyte cell death. While the use of AG490, a JAK2 inhibitor, eliminates the conditioning-induced cardioprotection [10]. These findings indicate the importance of activating the JAK2/STAT3 signaling pathway in improving the clinical outcomes of patients with I/R injury.

Pterostilbene (PTE), or trans-3,5-dimethoxy-4'-hydroxystilbene, is a dimethyl analog of resveratrol found naturally in grapes and blueberries and has superior bioavailability, high safety profile, and more potency compared to resveratrol [11,12]. This compound has recently attracted the attention of researchers due to its numerous benefits including anti-aging, anti-cancer, anti-diabetic, antioxidative, and neuroprotective effects. Previous studies have also reported the protective effects of PTE on the cardiovascular system. For example, PTE inhibited the growth of aortic vascular smooth muscle cells due to platelet-derived growth factor-B and the vascular cell cycle in mice through the Akt-dependent pathway [13]. PTE therapy has been able to reduce apoptosis due to I/R injury to cardiomyocytes by enhancing the activity of the phosphoinositide 3-kinase/Akt signaling pathway [14]. In addition, PTE protected atherosclerosis in rats by regulating the activity of the Toll-like receptor 4 (TLR-4)/NF-κB pathway [15].

In animal studies, PTE has 80% bioavailability compared to 20% for resveratrol, and thereby it is potentially considered a useful therapeutic agent [11]. On the other hand, the JAK2/STAT3 signaling pathway is a surviving anti-inflammatory and antioxidant pathway that plays a vital role

in the cardioprotective effects of conditioning interventions [8]. However, it remains unknown whether PTE can confer cardiac protection by activating this pathway. Therefore, this study aimed to investigate the effect of PTE on cardiac contractile and electrical function as well as inflammatory and oxidative responses in the setting of myocardial I/R injury and to discover the role of the JAK2/STAT3 pathway in the drug's cardioprotective effects.

Materials and Methods

Animals and Materials

Male 7-8 months old Sprague-Dawley rats weighing 230±25 g were purchased from Animal Center and allocated in the study. All animals were kept in the standard laboratory conditions with 22-24°C temperature and 50-55% humidity and a 12-hour darkness/lightness period. Rats had free access to food and water. This work was approved by the local ethical committee (JXPPH210419) and followed the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication). Pterostilbene was purchased from Sigma-Aldrich (St. Louis, MO, USA). ELISA kits and antibodies were obtained from MyBioSource Inc. and Cell Signaling Technology (CA, USA), and all other chemicals and reagents were attained from commercial sources in their highest quality available.

Experimental groups

The rats were randomly divided in six groups (n = 10 per group): Sham, I/R, I/R + PTE5 (I/R + receiving 5 mg/kg PTE, five min before reperfusion, intraperitoneally), I/R + PTE25 (I/R +

receiving 25 mg/kg PTE, five min before reperfusion, intraperitoneally), I/R + AG490, and I/R + AG490 + PTE25 (I/R + receiving 20 mg/kg AG490 at the onset of thoracotomy and 25 mg/kg PTE 5 min before reperfusion). AG490 was used to inhibit the JAK2/STAT3 pathway. Sham group experienced thoracotomy without induction of I/R injury. I/R group received the same amounts of saline as the drug vehicle [16]. After 24 h reperfusion, the electrical and contractile function of rats' hearts were measured, and then the animals were sacrificed for evaluation of myocardial infarct sizes (5 rats/group) and measurement of other parameters (5 rats/group).

Establishment of myocardial I/R injury

The animals were firstly anesthetized by sodium-pentobarbital (50 mg/kg; i.p). Then, a small animal ventilator (Harvard Apparatus, USA) was used to artificially ventilate the lungs of rats after intubation. Following heart exposure through an incision of intercostal muscles between the 4th and 5th ribs on the left side, the pericardium was detached and the left anterior descending (LAD) coronary artery was ligated using a 6.0 silk thread. The ligation was remained for 40 min to induce regional ischemia. Successful ischemia was approved by the discoloration of the left ventricles to pale. For initiation of reperfusion, the LAD occlusion was removed, and then the chest was sutured in layers with 2.0 silk thread. When rats were begun to try to breathe on their own, they are detached from the ventilator and returned to their cages. The reperfusion time was 24 h.

Measurement of myocardial function

At the end of reperfusion, the right carotid artery of rats was cannulated with a Millar pressure micro-catheter attached to a bridge pressure amplifier (AD-Instruments, Australia). After fixation in the place, the catheter was then advanced to the left ventricle. Heart rates (HR), Left ventricular end-diastolic pressure (LVEDP), left ventricular end-systolic pressure (LVESP), and left ventricular developed pressure (LVDP) were recorded at late reperfusion.

Recording and interpreting ventricular arrhythmias

Two golden recording electrodes and one neutral electrode were used for electrocardiography, using a data recording and acquisition system (Harvard apparatus, USA). Ventricular arrhythmias including premature ventricular complex (PVC), ventricular tachycardia (VT), and ventricular fibrillation (VF) were recorded during reperfusion, and their number and incidence were calculated and interpreted using the LAMBETH convention for animal arrhythmias (figure 1) [17].

Measurement of infarct size

To determine the infarct sizes of the hearts, the LAD coronary arteries were re-ligated at the end of reperfusion, and subsequently, 2-3 ml of 0.25% Evans blue was injected via the femoral vein and then the hearts were isolated, weighed, and stored at -20°C. The frozen hearts were cut into five ≈2 mm slices in the base-apical axis. The prepared slices were incubated in phosphate-buffered solution (PBS)-containing triphenyl-tetrazolium-chloride for 15 minutes at 37°C. Thereafter, Image J software (1.46r version, NIH, USA) was used to visualize the healthy and ischemic parts of the slices and to detect the left ventricular volumes, areas at risk, and infarct sizes. Infarct sizes were calculated as (area at risks/ventricular volumes)×100 for the hearts.

Measurement of pro-inflammatory mediators

After obtaining samples from the left ventricular ischemic region at the end of reperfusion, the samples were homogenized in RIPA buffer solution and after centrifugation and preparation of their supernatant, the contents of pro-inflammatory mediators (NF-κB, TNF-α, IL-1β) were detected using specific ELISA kits according to kit instructions (MyBioSource, Inc., USA). The protein content of the samples was also determined by the Bradford technique. The concentration of cytokines in each sample was standardized and reported based on the protein concentration of the samples.

Measurement of ROS production

Samples taken from the left ventricular ischemic regions were homogenized in RIPA buffer in the presence of protease inhibitor (Sigma-Aldrich, USA), and then centrifuged at 4,000 ×g at 4°C. For detecting cardiac ROS levels, the resulting supernatants were incubated for 30 minutes at 37 °C in PBS containing 2 μmol DCFDA dye (Sigma-Aldrich, USA). Then, using a fluorimeter, the amount of excitation and its emission were measured at 480 nm and 530 nm, respectively. The obtained values were calibrated based on the protein concentration of the samples and reported as ROS levels.

Measurement of cardiac endogenous antioxidants levels

The levels of endogenous antioxidants glutathione (GSH) and manganese superoxide dismutase (mnSOD) were measured spectrophotometrically. Left ventricular samples were homogenized and centrifuged at 4,000 ×g for 10 min at 4°C. The resultant supernatants were used to determine the antioxidant levels with assay kits, according to the instructions provided by the manufacturer (MyBioSource, Inc., USA). The activity of GSH and mnSOD was adjusted with sample proteins and reported as U/mg of protein.

Western blotting

After 24 h reperfusion, equal amounts (50 μg) of the resultant supernatants from peri-infarcted regions of left ventricles were electrophoresed in 10–15 % SDS-PAGE, and then the separated proteins were transferred to a polyvinylidene difluoride membrane (PVDF, Sigma-Aldrich, MO, USA). The membranes were incubated in Tris-buffered saline-Tween 20 (pH 7.4) containing 5% dry milk for 2 h at room temperature. After washing with PBS three times, membranes were incubated with primary antibodies against t-JAK2, p-JAK2, t-STAT3, p-STAT3, and GAPDH (1:1500, Cell Signaling Technology, USA). After overnight incubation, the membranes were washed again and exposed to secondary antibodies for 1 h. The enhanced-chemiluminescence reagents were used to create antibody-protein immunofluorescence reactions. Immunoblots were visualized and band intensities were calculated by Image J software and normalized with GAPDH as an internal control.

Statistical analysis

The data were reported as mean ± SD. After confirming the normal distribution of data, the differences between the groups were analyzed by one-way analysis of variance (ANOVA) and Tukey post hoc test. The minimum level of significance was considered at the alpha level of 0.05.

Results

PTE improved myocardial function and AG490 reversed its effect

The cardiac hemodynamic parameters in experimental groups were recorded at the end of reperfusion (Table 1). Induction of I/R injury in rats significantly reduced HR as compared with the sham group ($p<0.05$). The HR fluctuations were similar among the other treated groups. I/R injury significantly increased LVEDP ($p<0.01$), and reduced LVESP and LVDP ($p<0.05$) in comparison to the I/R group. Treatments of rats with PTE at the high dose (25 mg/kg) significantly diminished LVEDP and increased LVESP and LVDP ($p<0.05$). However, the effect of low-dose PTE (5 mg/kg) on the hemodynamic parameters was insignificant. Additionally, the protective effects of PTE on cardiac function (LVEDP and LVDP) were significantly reversed in rats pretreated with Ag490 as the inhibitor of JAK2, as compared with the I/R+PTE25 group ($p<0.05$).

PTE reduced ventricular arrhythmias and AG490 reversed its effect

The number of ventricular arrhythmias including PVC, VT ($p<0.01$), and VF ($p<0.001$) was significantly increased at early reperfusion following induction of I/R injury in rats (figure 2A-C). Administration of PTE significantly reduced the number of these arrhythmias in a dose-dependent manner. Their number in the I/R+PTE25 group was significantly lower than I/R ($p<0.001$) and

I/R+PTE5 ($p<0.05$) groups. Similarly, cardiac I/R induction significantly increased the incidence of ventricular arrhythmias ($p<0.01$ for PVC and VT, and $p<0.001$ for VF) (figure 2D). In I/R-treated rats, PTE at 5 mg/kg significantly reduced VT incidence ($p<0.01$), and at 25 mg/kg significantly reduced the incidence of PVC ($p<0.05$) and VT ($p<0.001$). Prior blockade of JAK2 by Ag490 in untreated I/R rats was not able to alter the number and incidence of arrhythmias, however, this blockade considerably abolished the antiarrhythmic features of PTE at 25 mg/kg on the number and incidence of ventricular arrhythmias ($p<0.05$ and $p<0.01$) (figure 2A-D).

PTE reduced cardiac infarct size and AG490 reversed its effect

The results of infarct size measurement (figure 3A-B) displayed that although there were no significant intergroup differences in the areas at risk of the I/R-injured hearts, induction of I/R significantly increased the infarct size as compared to sham rats ($p<0.001$). At low dose, PTE had no significant effect on infarct size, however, at high dose, it significantly reduced the infarct size in comparison to the I/R group ($p<0.01$). Its infarct size-limiting effect was also greater than low-dose PTE ($p<0.05$) (figure 3B). More importantly, prior treatment of rats with Ag490 (inhibition of JAK2/STAT3 pathway) significantly suppressed PTE's protective effect on cardiac infarct size ($p<0.05$).

PTE reduced cardiac pro-inflammatory mediators and AG490 reversed its effect

Following I/R insult, cardiac levels of inflammatory mediators NF-κB, IL-1β, and TNF-α were upregulated significantly compared to the sham rats ($p<0.01$) (figure 4A-C). Administration of PTE at 25 mg/kg significantly reversed the I/R-induced changes in inflammatory mediators

($p<0.01$). The effect of PTE at 25 mg/kg was also different from that of PTE at 5 mg/kg ($p<0.05$). Although Ag490 *per se* did not affect the parameters, it significantly abolished the anti-inflammatory impacts of PTE in I/R condition and increased the levels of NF-κB ($p<0.01$), IL-1β, and TNF-α ($p<0.05$) as compared with those of the I/R+PTE25 group.

PTE reduced oxidative stress levels and AG490 reversed its effect

The degree of oxidative stress among groups was quantified by measuring intracellular ROS production and the contents of GSH and mnSOD. The analysis showed that the level of intracellular ROS ($p<0.01$) was increased and the levels of GSH and SOD ($p<0.001$) were reduced following I/R injury induction (figure 5A-C). PTE dose-dependently reduced the I/R-induced oxidative stress in rats. Low dose PTE significantly diminished ROS level and augmented SOD level ($p<0.05$). However, high dose PTE significantly and strongly reduced ROS level ($p<0.01$) and increased GSH ($p<0.01$) and SOD ($p<0.001$) in comparison to the I/R group. Conversely, JAK2 pathway inhibition via Ag490 pretreatment significantly neutralized the effects of PTE on oxidative stress markers following cardiac I/R insult in rats ($p<0.05$ and $p<0.01$).

PTE upregulated cardiac JAK2/STAT3 phosphorylation and AG490 reversed its effect

Finally, the expression levels of JAK2 and STAT3 proteins in rat hearts were not significantly different between treated and untreated groups (figure 6B and 6D). However, I/R injury significantly downregulated the phosphorylated forms of JAK2 ($p<0.001$) and STAT3 ($p<0.01$) as compared to the I/R group (figure 6C and 6E). Treatment of rats with PTE, at 5 and 25 mg/kg, dose-dependently enhanced the activity of the JAK2/STAT3 pathway ($p<0.05$ to $p<0.001$), and

the effect of high dose was significantly greater than those of low dose ($p<0.05$). Importantly, pretreatment of rats with Ag490 significantly reversed the effects of PTE on the phosphorylation of JAK2 and STAT3 proteins as compared with the I/R+PTE25 group ($p<0.01$).

Discussion

In this study, we explored the involvement of the JAK2/STAT3 signaling pathway in the cardioprotective, anti-arrhythmic, anti-inflammatory, and antioxidative potentials of PTE following myocardial I/R injury in rats. Induction of I/R injury significantly led to increased myocardial dysfunction and increased myocardial infarct size, as well as the number and incidence of PVC, VT, and VT arrhythmias. Treatment of rats with PTE resulted in a significant reduction in infarct size and reperfusion-induced ventricular arrhythmic episodes. Additionally, PTE significantly downregulated the NF-κB, IL-1β, and TNF-α as the main inflammatory mediators and cytokines in I/R rats. It also reduced I/R-induced cardiomyocyte's ROS production and increased the antioxidant pools as indicated by the greater levels of GSH and SOD in PTE-treated rats. The beneficial effects of PTE were dose-dependent, so the greater protective effects were achieved in rats treated with PTE at 25 mg/kg. Importantly, inhibition of JAK2 considerably neutralized the protective features of PTE in I/R rats. Therefore, the treatment of rats with an appropriate dose of PTE can reduce the I/R-induced cardiac dysfunction and the occurrence of fatal arrhythmias through the activation of the JAK2/STAT3/antiinflammatory/antioxidative pathway.

During ischemia and subsequent reperfusion to the myocardium, several disturbances in cardiomyocytes' ionic currents, metabolic alterations, and biochemical dysregulations trigger

arrhythmogenic machinery [18,19]. Fatal ventricular arrhythmia is the main manifestation of reperfusion injury in patients with myocardial infarction [2,19]. Reperfusion-induced tachyarrhythmia deteriorates cardiac contractile function, leading to substantial cardiac dysfunction [2]. Abrupt induction of blood flow during reperfusion therapy endorses a cascade of cellular events including overproduction of ROS and oxygen free radicals, activation of the pro-inflammatory response, and imbalance in intracellular and intercellular homeostasis which eventually all facilitate the occurrence of VT and VF, resulting in enlargement of infarct size, heart failure and even cell death [20]. Therefore, a therapeutic strategy or drug that has antiarrhythmic effects in addition to beneficial effects will have more benefits in protecting the heart against I/R injury. In this line, PTE in the current study displayed a sufficient antiarrhythmic capacity besides its beneficial impacts on cardiac function recovery following reperfusion, and these effects of PTE were associated with strong cardioprotection in I/R rats.

There is ample evidence that oxidative stress and inflammatory reactions are major actors in the pathophysiology of myocardial I/R injury and their functions are closely related in this scene [4,5]. NF-κB signaling pathway serves a crucial contribution in the reciprocal recruitment of oxidative stress and inflammatory cytokines such as TNF-α in I/R condition [21,22]. TNF-α, in turn, encourages IKKb/NF-kB activity, by which it increases the release of other proinflammatory cytokines including IL-1β initiation of inflammatory responses [21]. Administration of PTE at high dosage significantly suppressed the activity of the NF-κB/TNF-α/IL-β pathway and positively modified the I/R-induced outcomes. Overproduction of ROS following I/R injury extremely provokes lipids peroxidation, proteins dysregulations, organelles dysfunction, and cardiomyocyte apoptotic death, thereby limiting the myocardial tolerance to ischemic insults [23]. In the present study, PTE had antioxidative properties at both concentrations and significantly restored I/R-

induced alterations in mnSOD and GSH activity as well as ROS levels. Consequently, the decrease in TNF-α and IL-1β levels, as well as the significant inhibition of oxidative stress after PTE administration can be attributed to the parallel reduction in NF-κB activity. These findings are consistent with the previous reports on the beneficial effects of PTE under various circumstances [13-16]. It has been reported that PTE inhibits neutrophil infiltration and inflammatory response during I/R injury in rat hearts [24]. In addition, PTE treatment had increased Bcl-2 to Bax expression ratio, indicating its antiapoptotic properties, and this protective effect of PTE was associated with activation of the PI3K/Akt signaling pathway [14]. Furthermore, suppressing oxidative stress and apoptosis via AMPK phosphorylation was another mechanism by which PTE attenuated hypoxia/reoxygenation injury in diabetic cardiomyocytes [16].

The important characteristic of the cardioprotective impacts of PTE in the present study was its potential to activate the JAK2/STAT3 signaling pathway. Indeed, blocking of this pathway through pre-administration of Ag490 completely attenuated the positive effects of PTE on cardiac function, ventricular arrhythmias, and inflammatory/oxidative responses. As a result, PTE-endorsed cardioprotection in rats with cardiac I/R injury potentially depends upon the activation of the JAK2/STAT3 pathway. JAK2/STAT3 is part of an intrinsic protective signaling program namely the survivor activating factor enhancement (SAFE) pathway to limit myocardial cell death [9,10]. This pathway mediates protective influences of ischemic conditioning or pharmacological strategies against reperfusion injury [8]. Upregulation of this critical pathway deals with the suppression of inflammatory, apoptotic, and oxidative events and thereby improves the electromechanical function of the hearts subjected to I/R injury [25]. Here, STAT3 is a multipotent transcription factor, upregulating the expression of different genes and contributing to the multiple cell surviving mechanisms [26]. The association of inhibition of STAT3 phosphorylation with the

all attenuated cardioprotective effects of PTE following Ag490 administration further confirms this conclusion. The association of STAT3 phosphorylation inhibition with attenuation of all cardioprotective effects of PTE after Ag490 administration further confirms this conclusion. Besides these pathways and mediators, more research is needed on how much PTE relies on non-JAK2/STAT3-related factors to produce its beneficial effects.

Conclusion

PTE exerted strong antiarrhythmic, infarct-sparing, and functional effects in the setting of myocardial I/R injury. The cardioprotective effects of PTE were accompanied by diminished levels of cardiac inflammatory and oxidative responses. Increased activity of JAK2/STAT3 may have a substantial contribution to PTE-induced cardioprotection. Thus, targeting JAK2/STAT3/NF-κB/TNF-α/ROS interaction may account for the greater effectiveness of PTE and be a reliable approach to reduce I/R injury outcomes.

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Conflict of interest: None.

Authors' Contribution: SL and YZ contributed to the study design and conception. SL and HW contributed to experiment conduction, data collection, and analysis. SL wrote the first draft of the manuscript. YZ authors critically revised the manuscript. All authors read and approved the final manuscript.

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Table 1. Cardiac hemodynamic parameters of the rats at the end of reperfusion.

Groups	HR (bpm)	LVEDP (mmHg)	LVESP (mmHg)	LVDP (mmHg)
Sham	275±39	7.4±1.3	104.1±12	98.6±10
I/R	241±33$^{\#}$	21.9±3.4$^{\#\#}$	83.6±9$^{\#}$	63.4±6$^{\#}$
I/R+PTE5	250±37	16.7±2.5	93.1±7	78.5±7
I/R+PTE25	258±29	11.6±2.0*	99.7±10*	86.0±6*
I/R+Ag490	282±42	22.3±3.8	86.3±6	66.3±4
I/R+Ag490+PTE25	264±40	17.0±1.6$^{@}$	87.3±7	70.1±5$^{@}$

Data of 10 rats in each group were presented as mean ± SD. HR: Heart rates, LVEDP: Left ventricular end-diastolic pressure, LVESP: Left ventricular end-systolic pressure, LVDP: left ventricular developed pressure, I/R: ischemia/reperfusion injury, PTE5 and PTE25: pterostilbene at 5 and 25 mg/kg respectively, and AG490: a JAK2 inhibitor. $^{\#}p<0.05$, and $^{\#\#}p<0.01$ vs. sham group; $^{*}p<0.05$ vs. I/R group; $^{@}p<0.05$ vs. I/R+PTE25 group.

Figure legends:

Figure 1. Tracing and classification of myocardial arrhythmias according to the Lambeth convention. Single, bigeminy and salvos beats are considered as premature ventricular complex (PVC).

Figure 2. Effect of PTE on the number and incidence of ventricular arrhythmias PVC, VT, and VF in rats with myocardial I/R injury. (A) PVC number; (B) VT number; (C) VF number; (D) arrhythmias incidence. Data of 10 rats in each group were presented as mean ± SD. PVC: premature ventricular complex; VT: ventricular tachycardia; VF: ventricular fibrillation; I/R: ischemia/reperfusion injury; PTE5 and PTE25: pterostilbene at 5 and 25 mg/kg respectively; and

AG490: a JAK2 inhibitor. $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. sham group; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. I/R group; $^{\$}p<0.05$ vs. I/R+PTE5 group; $^{@}p<0.05$, and $^{@@}p<0.01$ vs. I/R+PTE25 group.

Figure 3. Effect of PTE on infarct size in rats with myocardial I/R injury. (A) area at risk; (B) infarct size. Data of 5 rats in each group were presented as mean ± SD. I/R: ischemia/reperfusion injury; PTE5 and PTE25: pterostilbene at 5 and 25 mg/kg respectively; and AG490: a JAK2 inhibitor. $^{\#\#\#}p<0.001$ vs. sham group; $^{**}p<0.01$ vs. I/R group; $^{\$}p<0.05$ vs. I/R+PTE5 group; $^{@}p<0.05$ vs. I/R+PTE25 group.

Figure 4. Effect of PTE on inflammatory parameters in rats with myocardial I/R injury. (A) NF-κB; (B) IL-1β; (C) TNF-α. Data of 5 rats in each group were presented as mean ± SD. I/R: ischemia/reperfusion injury; PTE5 and PTE25: pterostilbene at 5 and 25 mg/kg respectively; and AG490: a JAK2 inhibitor. $^{\#\#}p<0.01$ vs. sham group; $^{**}p<0.01$ vs. I/R group; $^{\$}p<0.05$ vs. I/R+PTE5 group; $^{@}p<0.05$, and $^{@@}p<0.01$ vs. I/R+PTE25 group.

Figure 5. Effect of PTE on oxidative stress in rats with myocardial I/R injury. (A) intracellular ROS; (B) glutathione, GSH; (C) superoxide dismutase, SOD. Data of 5 rats in each group were presented as mean ± SD. I/R: ischemia/reperfusion injury; PTE5 and PTE25: pterostilbene at 5 and 25 mg/kg respectively; and AG490: a JAK2 inhibitor. $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. sham group; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. I/R group; $^{@}p<0.05$, and $^{@@}p<0.01$ vs. I/R+PTE25 group.

Figure 6. Effect of PTE on the activity of JAK2/STAT3 pathway in rats with myocardial I/R injury. (A) immunoblots; (B) total JAK2; (C) phosphorylated JAK2; (D) total STAT3; (E) phosphorylated STAT3. Data of 3 rats in each group were presented as mean ± SD. I/R: ischemia/reperfusion injury; PTE5 and PTE25: pterostilbene at 5 and 25 mg/kg respectively; and AG490: a JAK2 inhibitor. $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. sham group; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. I/R group; $^{\$}p<0.05$ vs. I/R+PTE5 group; $^{+}p<0.05$ vs. vs. I/R group; $^{@@}p<0.01$ vs. I/R+PTE25 group.

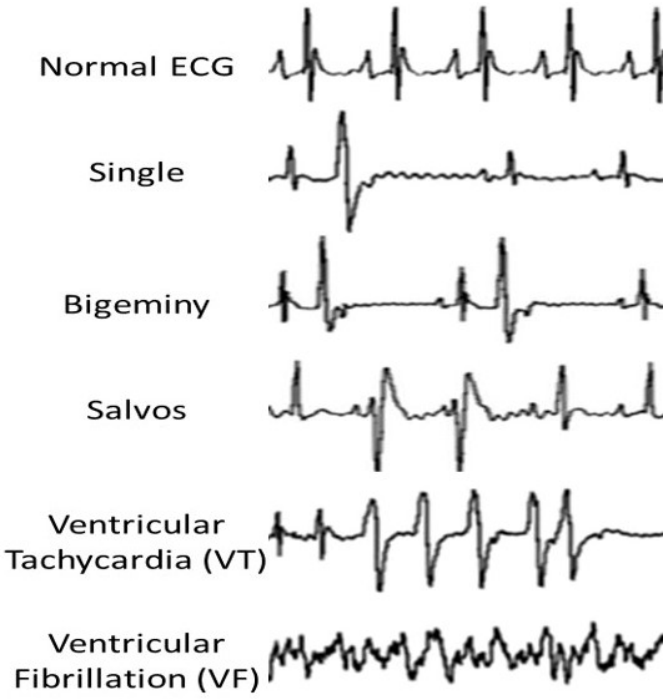
Normal ECG
Single
Bigeminy
Salvos
Ventricular Tachycardia (VT)
Ventricular Fibrillation (VF)

Figure 1.

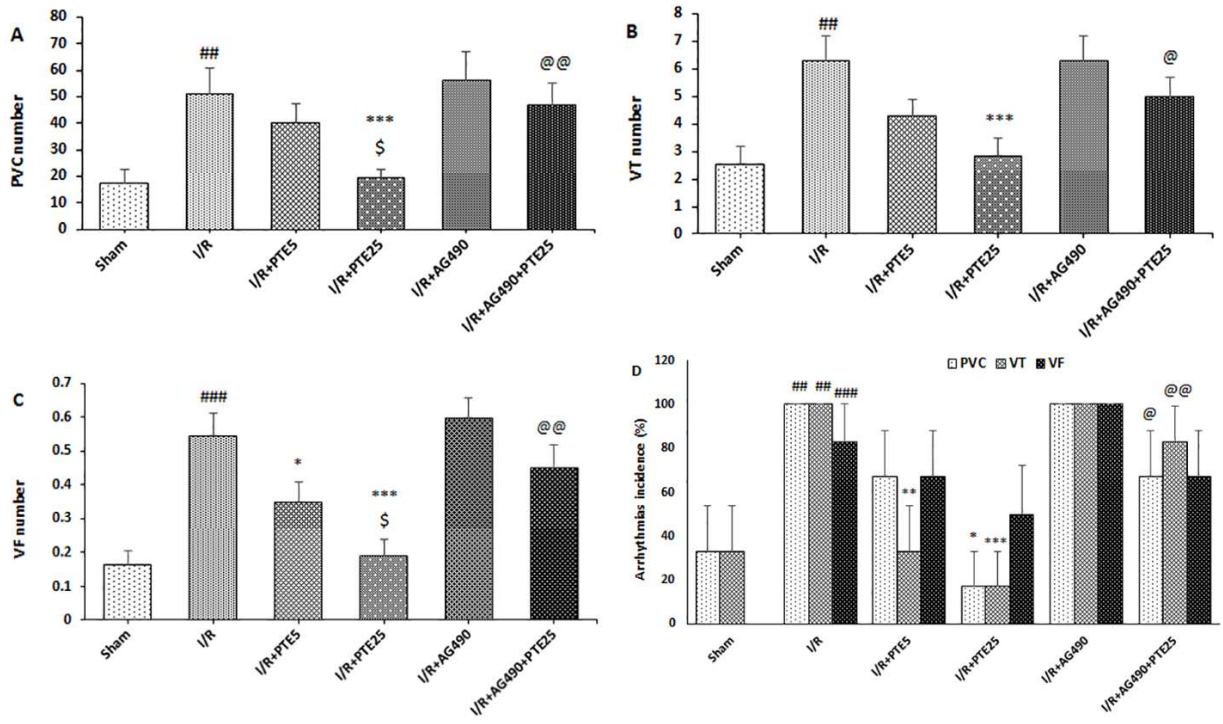

Figure 2.

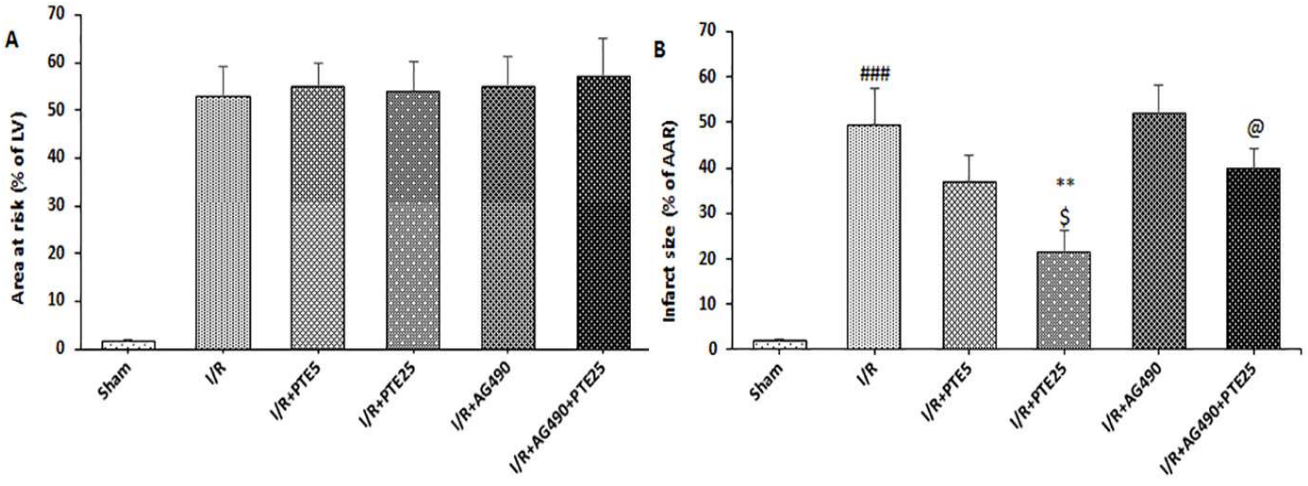

Figure 3.

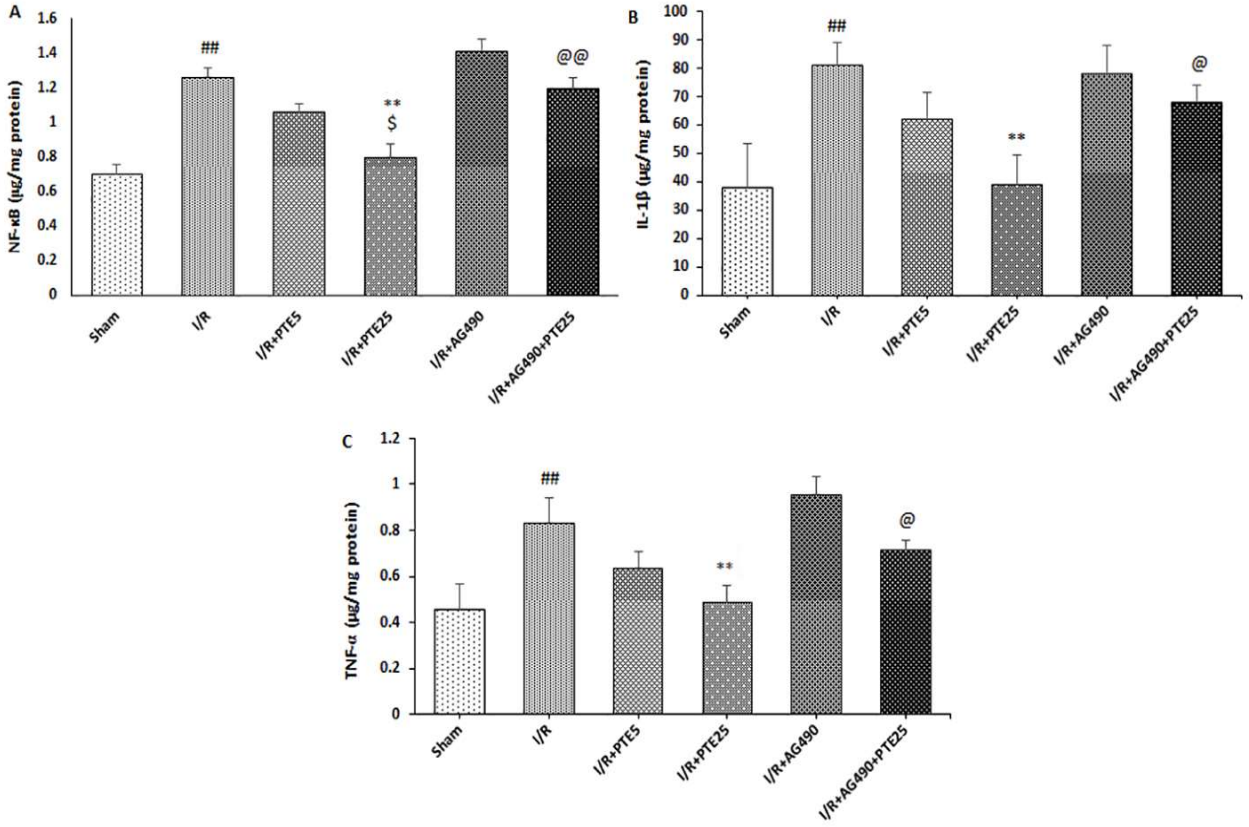
A
NF-κB (μg/mg protein)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
##
**
$
@@
Sham
I/R
I/R+PTE5
I/R+PTE25
I/R+AG490
I/R+AG490+PTE25
B
IL-1β (μg/mg protein)
100
90
80
70
60
50
40
30
20
10
0
##
**
@
Sham
I/R
I/R+PTE5
I/R+PTE25
I/R+AG490
I/R+AG490+PTE25
C
TNF-α (μg/mg protein)
1.2
1
0.8
0.6
0.4
0.2
0
##
**
@
Sham
I/R
I/R+PTE5
I/R+PTE25
I/R+AG490
I/R+AG490+PTE25

Figure 4.

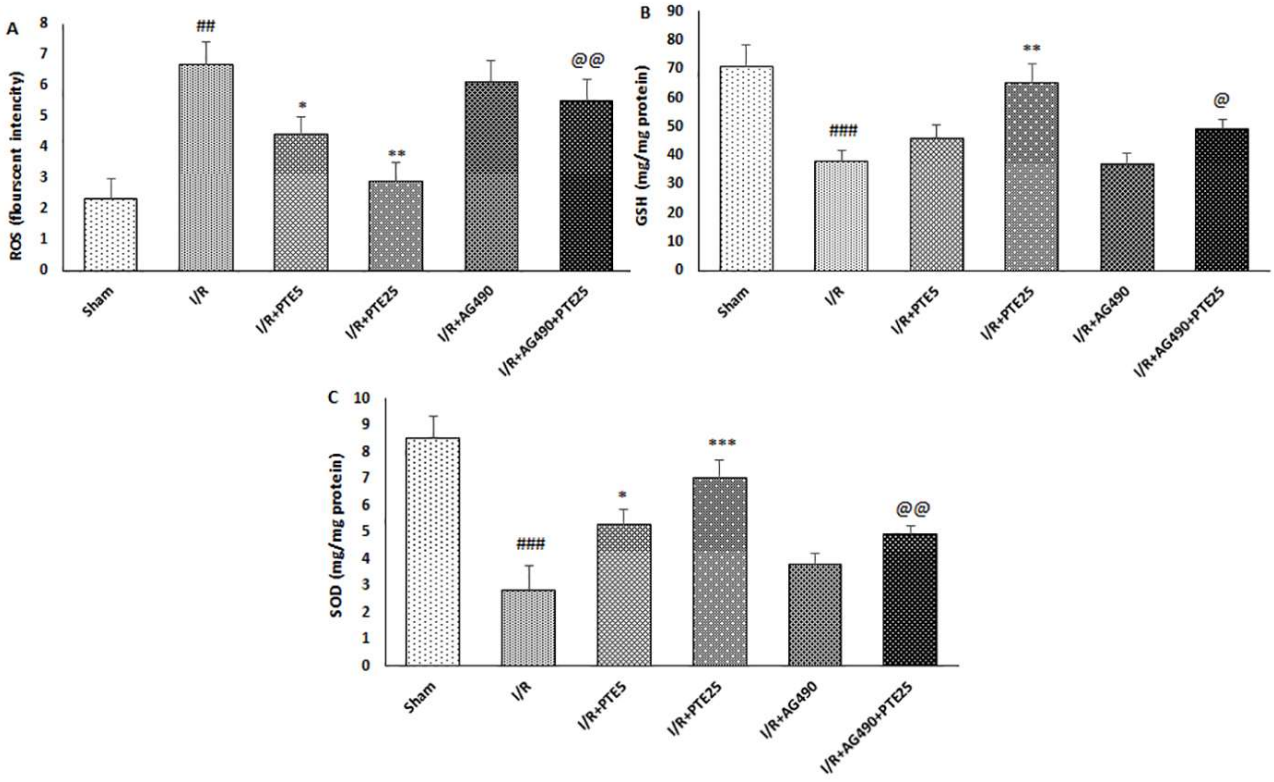
A
ROS (flourscent intencity)
0
1
2
3
4
5
6
7
8
##
*
**
@@
Sham
I/R
I/R+PTE5
I/R+PTE25
I/R+AG490
I/R+AG490+PTE25
B
GSH (mg/mg protein)
0
10
20
30
40
50
60
70
80
90
###
**
@
Sham
I/R
I/R+PTE5
I/R+PTE25
I/R+AG490
I/R+AG490+PTE25
C
SOD (mg/mg protein)
0
1
2
3
4
5
6
7
8
9
10
###
*
***
@@
Sham
I/R
I/R+PTE5
I/R+PTE25
I/R+AG490
I/R+AG490+PTE25

Figure 5.

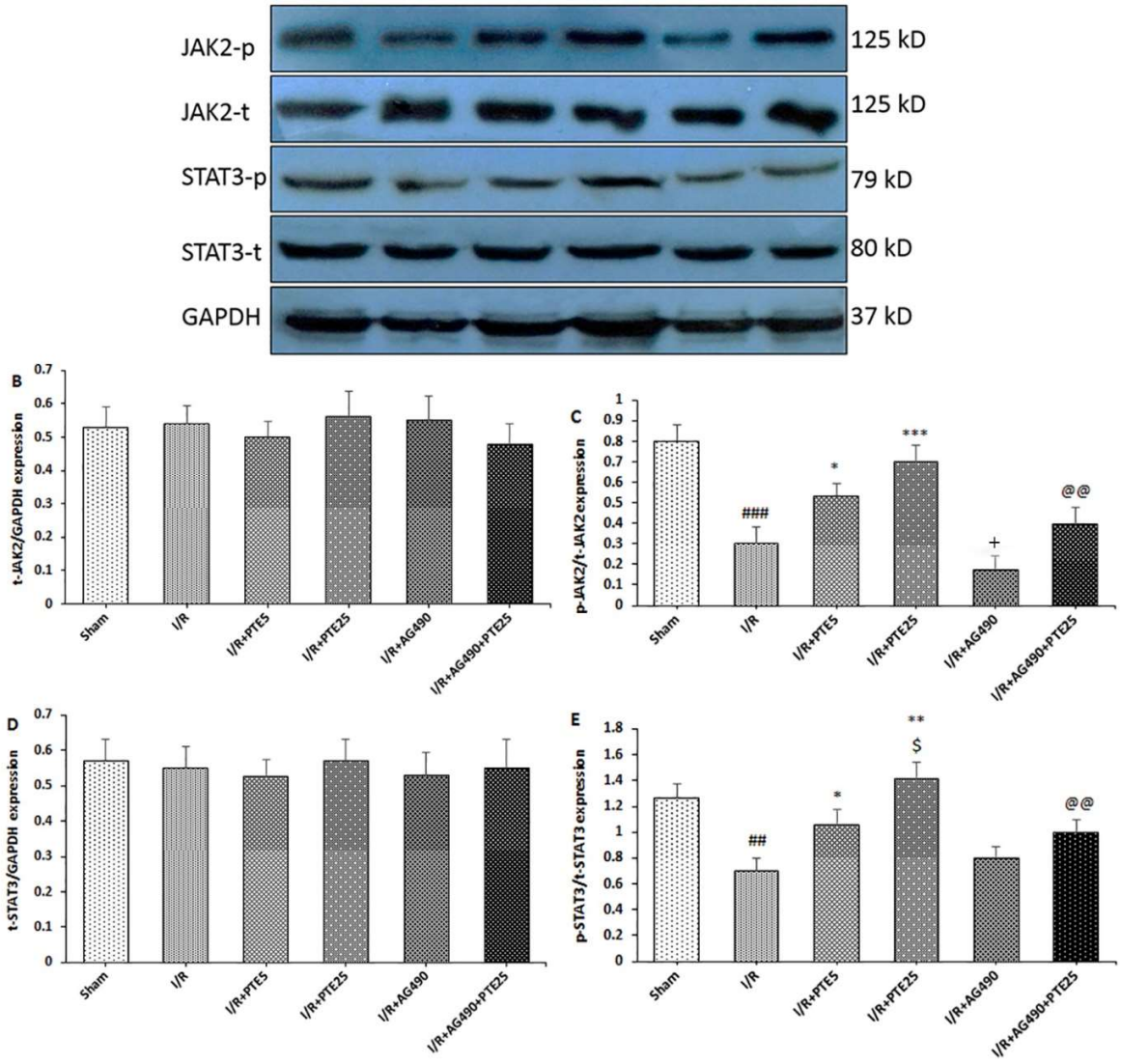
JAK2-p
JAK2-t
STAT3-p
STAT3-t
GAPDH
125 kD
125 kD
79 kD
80 kD
37 kD
B
t-JAK2/GAPDH expression
C
p-JAK2/t-JAK2expression
D
t-STAT3/GAPDH expression
E
p-STAT3/t-STAT3 expression
Sham
I/R
I/R+PTE5
I/R+PTE25
I/R+AG490
I/R+AG490+PTE25

Figure 6.