The Effects of Progranulin in a Rat Model of Acute Myocardial Ischemia/Reperfusion are Mediated by Activation of the P13K/Akt Signaling Pathway

Authors' Contribution:
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Data Collection B
Statistical Analysis C
Data Interpretation D
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Background: Progranulin is an adipokine, encoded by the progranulin (GRN) gene. Progranulin is expressed in atherosclerosis, but its effects in cardiac ischemia and reperfusion injury are unknown. Therefore, this study aimed to investigate the effects of progranulin in a rat model of acute myocardial ischemia/reperfusion (MI/R) injury *in vivo*.

Material/Methods: The model of acute MI/R injury was established in male Wistar rats by ligation of the left anterior descending (LAD) coronary artery for 30 minutes and reperfusion for 60 minutes. Before modeling, one group was treated with progranulin (0.03 µg/kg), and one group was treated with the P13K/Akt inhibitor, LY294002 (3 mg/kg). Left ventricular function (LV) was monitored, including the LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), and changes in LV pressure. At the end of the study, blood and myocardial tissue were examined. Cardiac biochemical markers, histopathology, gene expression, and apoptosis were analyzed.

Results: Progranulin improved cardiac function following acute MI/R injury and significantly improved recovery of cardiac contractility and LVSP. Progranulin significantly reduced myocyte apoptosis, inflammation, and tissue edema, and was highly expressed in cardiac tissue following MI/R injury. The cardioprotective effect of progranulin was reduced by blocking the P13K/Akt signaling pathway.

Conclusions: In the rat model of acute MI/R injury, progranulin had a protective effect on cardiac function and morphology, associated with activation of the P13K/Akt signaling pathway. The mechanisms of the anti-apoptotic, anti-inflammatory, and inotropic effects of progranulin in the setting of acute MI/R injury require further *in vivo* studies.

MeSH Keywords: Adipokines • Atherosclerosis • Myocardium • Reperfusion Injury

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Background

Worldwide, ischemic heart disease is a major cause of death [1]. Obesity is a risk factor for ischemic heart disease [2]. Adipose tissue secretes growth factors or adipokines, which also have roles in cardiac function [3]. Several adipokines have shown cardioprotective properties, while others are associated with cardiac disease [4]. The adipokines that include adiponectin and several of the C1q/tumor necrosis factor (TNF)-related proteins (CTRPs) have favorable actions in the vasculature and heart [4,5]. Adiponectin has a protective role in atherosclerosis and myocardial ischemia/reperfusion (MI/R) injury by exerting anti-inflammatory, antioxidant, anti-apoptotic, and antithrombotic effects [4,6–8]. The CTRPs have shown protective effects against myocardial ischemia/reperfusion (MI/R) injury and cardiac remodeling by anti-apoptotic mechanisms [9,10]. Cardioprotective adipokines have been shown to exert their anti-apoptotic effects through activation of the P13K/Akt survival pathway [9,10]. The PI3-K/AKT signaling pathway and downstream Akt kinase are important regulators of cell survival and proliferation and play an important role in the prevention of cell apoptosis [11,12].

Progranulin is an adipokine that was initially recognized as a growth factor [13]. Progranulin has roles in cell growth and wound healing, and is encoded by the progranulin (GRN) gene [14–16]. In human disease, progranulin expression has been shown in tumorigenesis [14], and degenerative conditions of the nervous system [17]. Progranulin also has anti-inflammatory activity [18,19], and previous studies have shown that progranulin could reduce acute lung injury, inflammatory arthritis, and inflammatory bowel disease [20–22]. However, in diabetes and obesity, progranulin levels are increased and have been reported to be associated with proinflammatory activity [23]. In the cardiovascular system, progranulin is highly expressed in vascular smooth muscle cells and macrophages in atherosclerotic arteries [18]. Due to the increasing incidence of obesity and its association with ischemic heart disease, identifying cardioprotective adipokines has recently gained research attention. Not only is progranulin involved in atherosclerosis, which is the main cause of myocardial ischemia and infarction, it has also been shown to enhance cardiac contractility [24]. However, its effects in acute myocardial ischemia and reperfusion injury are unknown. Therefore, this study aimed to investigate the effects of progranulin in a rat model of acute myocardial ischemia/reperfusion (MI/R) injury *in vivo*.

Material and Methods

Animals

The study protocol was approved by the Institutional Review Board of the Faculty of Medicine, King Saud University.

Male albino Wistar rats weighing between 250–300 gm were supplied by the Animal House, College of Medicine, King Saud University. The rats were maintained in standard conditions with an ambient temperature of between 21–23°C, with a 12-hourly light and dark cycle, and were fed standard chow, with free access to tap water.

The rat model of acute myocardial ischemia/reperfusion (MI/R) injury and monitoring of cardiac function

Drugs and anesthesia (urethane 1.25 g/kg) were administered by intraperitoneal injection. The rats underwent *in vivo* acute MI/R injury by ligation of the left anterior descending coronary artery for 30 minutes and reperfusion for 60 minutes [25].

Study groups

The rats (n=6–8 in each group) were randomly divided into the following four groups: the sham group, in which the rats underwent the surgical procedure without coronary artery occlusion; the IR group, which included rats in the model of acute myocardial ischemia/reperfusion (MI/R) injury, without treatment; the progranulin/IR group, in which the rats in the model were treated with progranulin before MI/R; and the LY/progranulin/IR group that were treated with the PI3K/Akt signaling pathway inhibitor, LY294002, and progranulin before acute MI/R injury. The rats treated with progranulin received two doses of progranulin 0.03 μg/kg, 24 hours apart. Human recombinant progranulin was obtained from R&D Systems (Minneapolis, MN, USA), and the assigned doses were selected according to our previous pilot studies (*unpublished data*). The PI3K/Akt signaling pathway inhibitor LY294002 (3 mg/kg) (BioSource/Thermo Fisher Scientific, Waltham, MA, USA) was used to block the PI3K/Akt signaling pathway.

Cardiac function parameters

Left ventricular function was monitored in the rat study groups, including left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and the maximal rates of increase and decrease in left ventricular pressure (±dp/dtmax). At the end of each experiment, myocardial tissue was snap-frozen in liquid nitrogen and stored at –80°C for further analysis. Cardiac tissue samples were preserved in 10% buffered formalin for histological studies.

Measurement of serum cardiac enzymes

After completion of the ischemia/reperfusion model, blood samples were collected from the retro-orbital sinus and centrifuged at 5000 rpm for 5 minutes at 4°C. Serum cardiac enzymes, including creatine kinase (CK) and lactate dehydrogenase (LDH) levels were determined using Siemens Dimension®

diagnostic kits, Siemens Dimension® Integrated Chemistry System, and Siemens, Dimension® RxL Max Integrated Chemistry System (Siemens Healthcare, Washington, DC, USA) in the Clinical Laboratory of King Khalid University Hospital.

Histology and histochemical staining with periodic acid Schiff (PAS)

At the end of the study, rat cardiac tissue were immediately excised and fixed in 10% neutral-buffered formalin, processed, embedded in paraffin wax, sectioned, and stained with periodic acid-Schiff (PAS) for histology.

Immunostaining for the apoptosis marker (p53) and the anti-apoptosis marker (Bcl-2)

The rat cardiac tissues that were embedded in paraffin wax were sectioned and stained with Confirm anti-p53 (DO-7) and anti- Bcl-2 (SP66) rabbit monoclonal primary antibodies (Ventana-Roche Medical Systems, Tucson, AZ, USA). The immunostaining of the tissue sections was performed using the UltraView DAB Detection Kit and the Benchmark XT automated immunostaining system (Ventana, Tucson, AZ, USA).

Immunostaining for progranulin

Immunohistochemical staining was performed on formalin-fixed paraffin tissue sections of rat cardiac tissue. Progranulin was detected using the acrogranin (H-300) antibody (sc-28928) (1: 100) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Immunohistochemistry was performed using the UltraView DAB Detection Kit and the Benchmark XT automated immunostaining system (Ventana, Tucson, AZ, USA).

Western blot

Protein was extracted from the rat cardiac tissue. Liquid nitrogen was used to rapidly freeze the sample, which was powdered and placed in 300 µl of RIPA lysis buffer (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The samples were sonicated using a Vibra-Cell Ultrasonic Processor (Sonics & Materials Inc., Newtown, CT, USA) to homogenize the ground tissue, which was centrifuged twice for 15 minutes at 10,000×g at 4°C. The supernatant was collected, and the protein concentration was assessed using the Bradford protein assay (Sigma-Aldrich, St. Louis MO, USA). Protein homogenate samples (150 µg) were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, then transferred to nitrocellulose membranes 0.45 µm (Bio-Rad, Hercules, CA, USA), and blocked with 5% dried skimmed milk powder prepared in Tris-buffered saline containing Tween 20 (TBST). The membranes were incubated overnight at 4°C with a primary antibody to progranulin (1: 300) (acrogranin)

(H-300, sc-28928) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), and beta-actin (1: 1000) (ab8227) (Abcam, Cambridge, MA, USA). After four washes in TBST, the membranes were incubated with a secondary goat anti-rabbit horseradish peroxidase (HRP) conjugated IgG antibody (1: 10000) (sc-2004) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for 1 hour at room temperature. Signals were detected with an enhanced chemiluminescence (ECL) kit (sc-2048) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The expression levels were determined by measurement of the corresponding band intensities using ImageJ2 software from the National Institutes of Health (NIH) (Bethesda, MD, USA).

Real-time reverse transcription-polymerase chain reaction (RT-PCR)

Real-time RT-PCR quantified the mRNA level of the progranulin gene, GRN, in the rat cardiac tissue. RNA extraction from the rat cardiac tissue was prepared with the RNeasy Protect Mini Kit (Qiagen, Inc., Hilden, Germany) according to the manufacturer's instructions. RNA concentration and purity were determined on a spectrophotometer Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). For the Real-Time One-Step RT-PCR procedure, Rotor-Gene Multiplex PCR Kit (Qiagen, Inc., Hilden, Germany) was used according to the manufacturer's instructions. The primers and probes for progranulin and Beta-actin (Eurofins Genomics, Luxembourg) were used with the following primer sequences:

Rat progranulin (GRN), forward: 5'-CTGGGAAGTATGGCTGTTGT-3';
Rat progranulin (GRN), reverse: 5'-CTGTGGTGTAGTCCTTGGATATG-3';
Beta-actin, forward: 5'-GTGTGGATTGGTGGTCTATC-3';
Beta-actin, reverse: 5'-CAGTCCGCCTAGAAGCATTT-3';
Rat progranulin (GRN), Taqman probe: 5'-CAATGCCCAACGCCATCT-3' (5'-Hex, 3'-BHQ1);
Beta-actin, Taqman probe: 5'-ACTGTCCACCTTCCAGCAGATGTG-3' (5'-FAM, 3'-BHQ1).

The TaqMan probes included fluorescent reporter dyes (FAM or HEX) attached to the 5' end, while the Quencher BHQ1 (for FAM and HEX) was attached to the 3' end. Each reaction volume contained 8.75 µl of RNase-free water, 12.5 µl of 2×Rotor-Gene Multiplex RT-PCR master mix (Qiagen, Inc., Hilden, Germany), 1.25 µl of 20x primer-probe mix, 0.25 µl Rotor-Gene RT Mix, and 1 µl of template DNA. For RT-PCR amplification of progranulin, an initial amplification using progranulin primers was performed with a denaturation step at 95°C for 5 minutes, followed by 45 cycles of denaturation at 95°C for 15 sec, primer annealing extension at 60°C for 15 sec. On completion of the cycling steps, a final extension at 95°C for 5 minutes was performed, and then the reaction was stored at 4°C. Real-time PCR was performed on a Rotor-gene Q Real-Time PCR Detection system. (Qiagen, Inc., Hilden, Germany). Expression levels were obtained using the ΔΔct relative quantification method.

Reactions were run in triplicate in independent experiments, and the resulting data were analyzed using the Rotor-Gene Q Series software version 2.1.0 (Qiagen, Inc., Hilden, Germany).

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 21 (SPSS Inc., Chicago, IL, USA), and data were expressed as the mean±standard deviation (SD). One-way analysis of variance (ANOVA) was used for normally distributed data followed by post hoc tests for multiple comparisons. The Kruskal-Wallis test was used for comparisons between groups for data not normally distributed, and the Mann-Whitney test was applied to find the significant difference between any two groups. A P-value <0.05 was considered to be statistically significant.

Results

The effect of progranulin administration on myocardial function in the four study groups of rats

Four study groups included the sham group; the IR group, which included rats in the model of acute myocardial ischemia/reperfusion (MI/R) injury, without treatment; the progranulin/IR group, in which the rats in the model were treated with progranulin before MI/R; and the LY/progranulin/IR group that were treated with the PI3K/Akt signaling pathway inhibitor, LY294002, and progranulin before acute MI/R injury.

The effect of progranulin on acute myocardial ischemia in the rats was evaluated by determining the recovery of heart function after *in vivo* induced myocardial ischemia/reperfusion (MI/R) injury. Cardiac function improved significantly in the group that received progranulin before MI/R injury. This finding was demonstrated by the increased recovery of cardiac contractility (±dp/dtmax) in the rats treated with progranulin. The progranulin/IR group showed significant recovery of ±dp/dtmax, with an 80% change (P<0.001) and left ventricle systolic pressure (LVSP), with a 53% change (P<0.001) when compared with the progranulin/IR group (Figure 1A–1C). Also, treatment with progranulin resulted in the recovery of cardiac function following I/R, as shown by a significantly lower LV end-diastolic pressure (LVEDP) (P<0.04) (Figure 1D). Also, when the PI3K/Akt signaling pathway was inhibited, the group that received progranulin showed similar myocardial contractility to the ischemic group, ±dp/dt$_{max}$ (P<0.2) indicating that the effect of progranulin was reduced by blocking the PI3K/Akt signaling pathway (Figure 1A–1D).

The effect of progranulin administration on cardiac enzymes in the rat model of acute MI/R injury

MI/R injury resulted in significantly increased levels of cardiac enzymes when compared with the sham group, including creatine kinase (CK) (P<0.001) and lactate dehydrogenase (LDH) (P<0.004). However, the administration of progranulin significantly reduced the rise of CK levels (P<0.001) (Figure 2A) and LDH levels (P<0.004) (Figure 2B) in rats after MI/R injury compared with the sham group.

The effect of progranulin administration on the histology of the cardiac tissue in the rat model of acute MI/R injury

In the ischemic region of the myocardium in the progranulin/IR group, edema of myocardial fibers, congestion, and vasodilatation were present when compared with the sham. The administration of progranulin reduced the I/R-induced myocardial congestion and edema (Figures 3A–3C).

The effect of progranulin administration on MI/R injury-induced apoptosis

The administration of progranulin increased the expression of the anti-apoptotic protein (Bcl-2) in rat cardiac tissue exposed to I/R injury, while untreated rats did not express Bcl-2 following induction of MI/R injury (Figure 4A–4C). However, the p53 apoptotic protein was not expressed after MI/R injury in all groups.

The expression of progranulin in cardiac tissue following myocardial ischemia/reperfusion injury

The presence of progranulin in the rat cardiac tissue following MI/R injury was assessed by immunohistochemistry staining with the progranulin antibody. Progranulin protein was detected in the myocardium after ischemia/reperfusion, whereas no progranulin expression could be detected in the cardiac tissue of sham rats. After treatment with progranulin and induction of MI/R injury, progranulin expression was increased (Figure 5A–5C).

Myocardial expression of progranulin in MI/R injury

Western blot analysis detected progranulin protein in the cardiac tissue after MI/R injury. The expression of progranulin was significantly increased in the MI/R group when compared with the sham group (P<0.008) (Figure 6A, 6B).
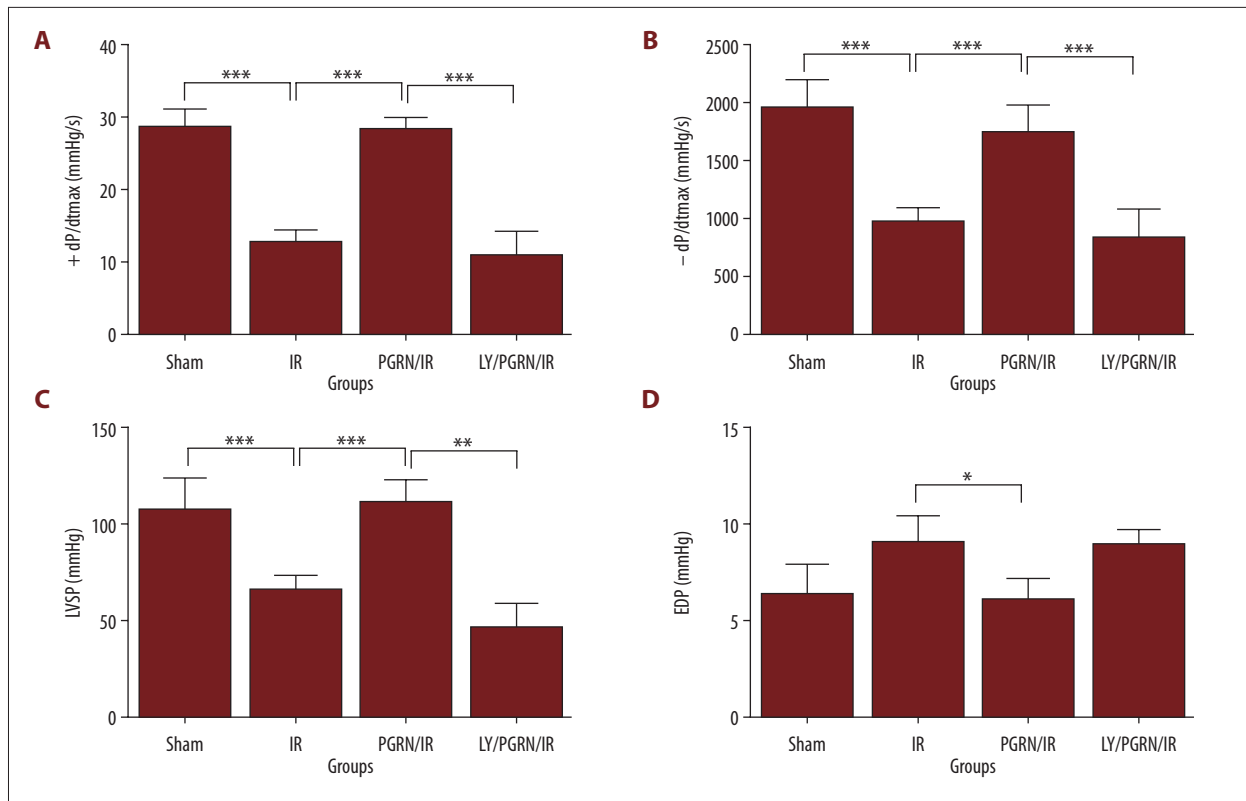
Figure 1. The administration of progranulin improved cardiac function in the rat model of acute myocardial ischemia/reperfusion (MI/R) injury. The effects of progranulin on cardiac function are shown by the improvement in the maximum and minimum rate of rise in left ventricular pressure (**A, B**), left ventricular systolic pressure (**C**), and end-diastolic pressure (**D**). Blocking the PI3K/AKT signaling pathway reduced the effect of progranulin. Data are expressed as the mean±SD (n=6–8 in each group). I/R – ischemia/reperfusion; PGRN – progranulin; LVSP – left ventricular systolic pressure; EDP – end-diastolic pressure; +dp/dt max – maximum rate of rise in left ventricular pressure; –dp/dt max – minimum rate of rise in left ventricular pressure; LY – PI3K/AKT signaling pathway blocker (LY294002). * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ indicate statistical significance.
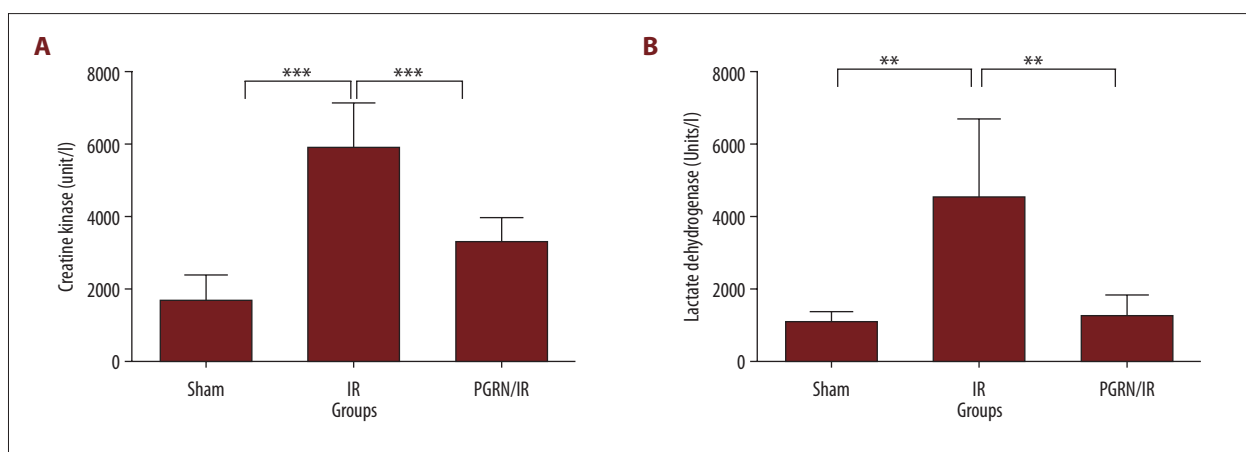

Figure 2. The administration of progranulin reduced the rise in serum levels of cardiac enzymes in the rat model of acute myocardial ischemia/reperfusion (MI/R) injury. Following treatment with progranulin, reduced levels of the cardiac enzymes are shown, including creatine kinase (CK) (**A**) and lactate dehydrogenase (LDH) (**B**) in myocardial ischemia/reperfusion (MI/R) injury in rats (n=6 in each group). I/R – ischemia/reperfusion; PGRN – progranulin. ** $P<0.01$ and *** $P<0.001$ indicate statistical significance.
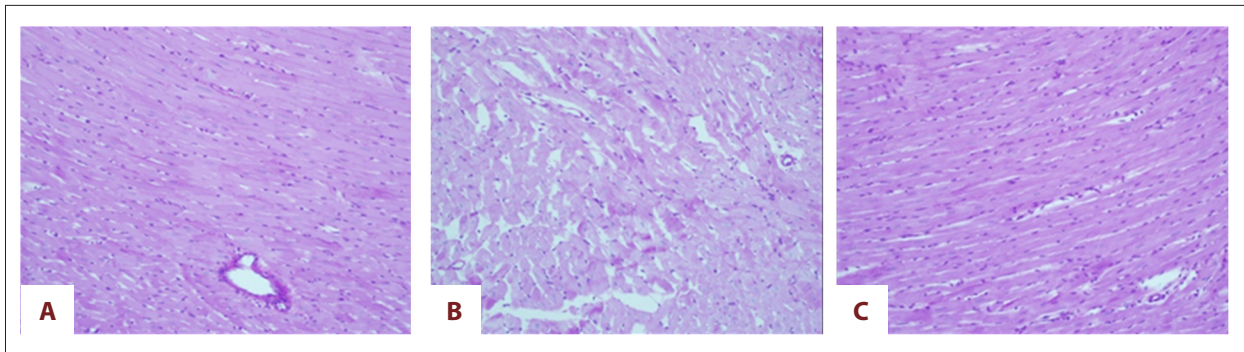
Figure 3. Representative photomicrograph images of the histology of the left ventricle in the sham and the rat model of acute myocardial ischemia/reperfusion (MI/R) injury. Periodic acid-Schiff (PAS) staining of cardiac tissue in the sham group (**A**) and the acute myocardial ischemia/reperfusion (MI/R) injury group (**B**). The administration of progranulin reduced congestion, edema, and inflammation in cardiac tissue after ischemia/reperfusion injury in the rat model (**C**). I/R – ischemia/reperfusion; PGRN – progranulin. Magnification ×200.
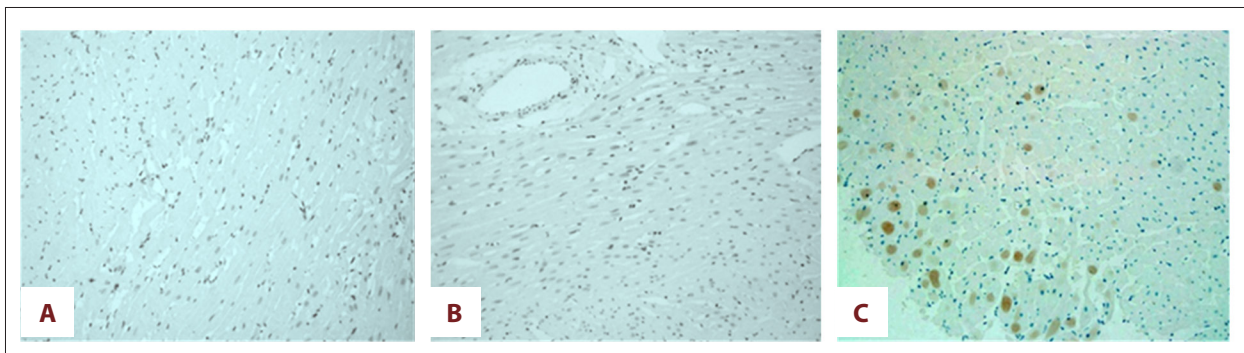

Figure 4. Photomicrographs of the immunohistochemistry staining of rat cardiac tissue for the anti-apoptotic protein, Bcl-2, in cardiac tissue in the sham and the rat model of acute myocardial ischemia/reperfusion (MI/R) injury. Immunohistochemistry for Bcl-2 in the sham (**A**) and the rat model of acute myocardial ischemia/reperfusion (MI/R) injury (**B**). The administration of progranulin increased the expression of Bcl-2 in acute MI/R injury in the rats (**C**). Magnification ×400.

Figure 5. Photomicrographs of the immunohistochemistry staining of rat cardiac tissue for progranulin in the sham and the rat model of acute myocardial ischemia/reperfusion (MI/R) injury. Progranulin could not be detected in the cardiac tissue of the rats in the sham group (**A**). The expression of progranulin was detected in the myocardium following acute myocardial ischemia/reperfusion (MI/R) injury (**B**). After treatment with progranulin and induction of ischemia/reperfusion, progranulin expression was increased (**C**). Magnification ×400.
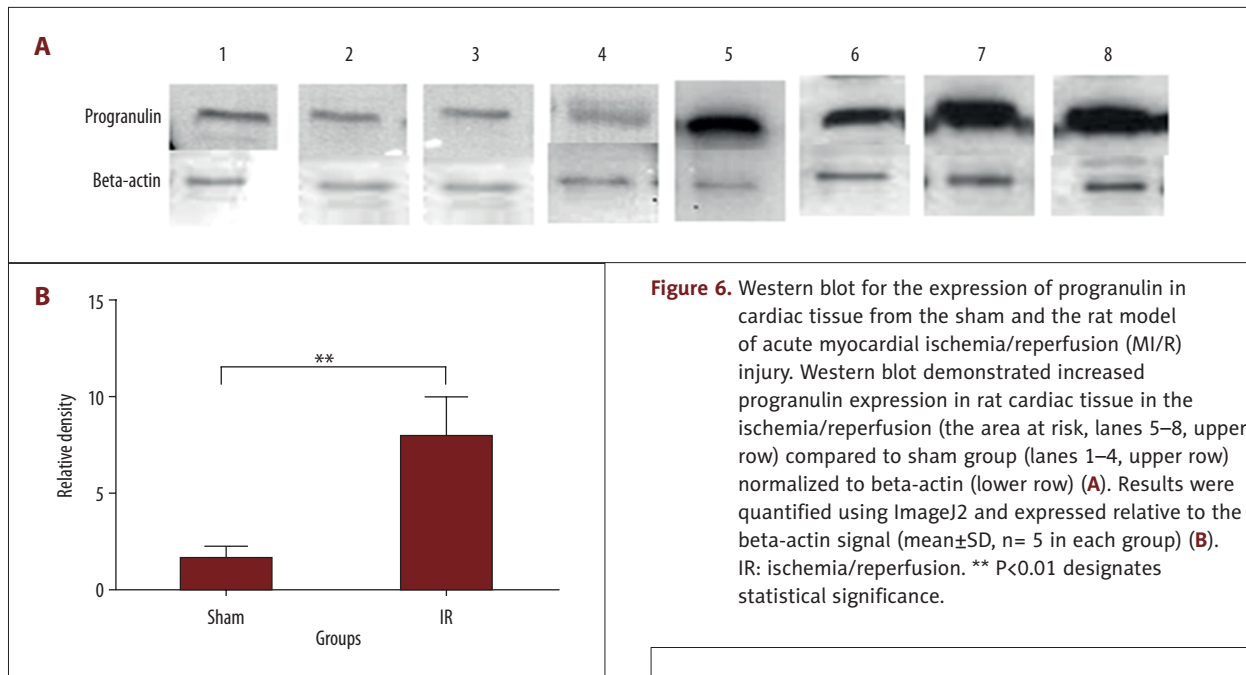
Figure 6. Western blot for the expression of progranulin in cardiac tissue from the sham and the rat model of acute myocardial ischemia/reperfusion (MI/R) injury. Western blot demonstrated increased progranulin expression in rat cardiac tissue in the ischemia/reperfusion (the area at risk, lanes 5–8, upper row) compared to sham group (lanes 1–4, upper row) normalized to beta-actin (lower row) (**A**). Results were quantified using ImageJ2 and expressed relative to the beta-actin signal (mean±SD, n= 5 in each group) (**B**). IR: ischemia/reperfusion. ** P<0.01 designates statistical significance.

Expression of the progranulin (GRN) gene following myocardial ischemia/reperfusion injury

Myocardial ischemia/reperfusion injury resulted in increased progranulin (GRN) mRNA levels in the cardiac tissue (1.3–2.4 fold). The change in fold expression after MI/R injury was significantly increased when compared with the sham group (*P*<0.01) (Figure 7).

Discussion

The findings from the present study showed that in a rat model of acute myocardial ischemia/reperfusion (MI/R) injury the administration of progranulin improved cardiac function, reduced inflammation, protected the heart from injury with an anti-apoptotic effect, and progranulin expression was upregulated in cardiac tissue following MI/R injury. The use of the P13K/Akt inhibitor, LY294002, showed that progranulin exerted its cardioprotective effect through activating the PI3K/Akt signaling pathway.

Progranulin is an adipokine, encoded by the progranulin (GRN) gene, which is expressed in atherosclerosis. In atherosclerosis, adipokines may inhibit atherogenesis and have a preventive or protective role in ischemic heart disease [3]. In the GRN knockout mice that develop atherosclerosis associated with increased expression of inflammatory cytokines and adhesion molecules and a reduction in endothelial nitric oxide (NO), progranulin has shown antiatherogenic effects [26]. Also, progranulin enhances the protection of the vascular endothelium by
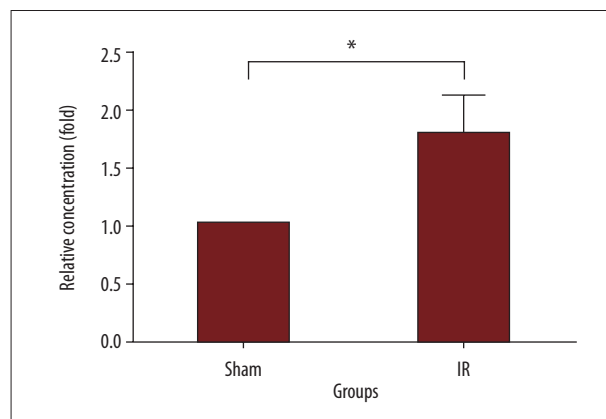

Figure 7. Real-time reverse transcription-polymerase chain reaction (RT-PCR) for the expression of the mRNA of the progranulin (GRN) gene in cardiac tissue from the sham and the rat model of acute myocardial ischemia/reperfusion (MI/R) injury. Progranulin mRNA levels increased (1.3-2.4) fold in rat cardiac tissue (the area at risk) subjected to acute myocardial ischemia/reperfusion (MI/R) injury compared with the sham, (n=4 in each group). I/R, ischemia/reperfusion. * P<0.05 indicates statistical significance.

increasing NO through activation of the Akt/eNOS signaling pathway [27]. Progranulin secreted from macrophages forms a complex with apolipoprotein A-I, which may play a role in stabilizing the atherosclerotic plaque [28]. Lack of progranulin changes the configuration of high-density lipoprotein (HDL), enhancing pro-inflammatory activity in atherosclerotic lesions, and progranulin reduces the level of platelet-activating factor acetylhydrolase, an HDL-associated antioxidant molecule [26], indicating that progranulin might reduce the development of macrophage foam cells and inhibit atherogenesis [29]. However,

the role of progranulin in acute MI/R injury in a rat model has not been previously studied.

The findings from the present study showed that progranulin treatment of the rat model of acute MI/R injury resulted in significant recovery of cardiac performance. Progranulin reversed the effects of ischemia on cardiac function and reduced cellular damage. Several cardioprotective adipokines, including adiponectin, CTRP3, and CTRP9, have shown similar effects in previous studies [6,7,9,10]. Moreover, in this study, treatment with progranulin reduced acute inflammation, edema, vasodilatation, and congestion in cardiac tissue following acute MI/R injury. In ischemia/reperfusion injury of the brain, progranulin was shown to reduce inflammation by inhibiting TNF-α mediated neutrophil infiltration and the expression of cell adhesion molecules [30]. In renal tissue, progranulin reduces hypoxia-induced inflammation [31]. Progranulin may inhibit inflammation in acute MI/R injury in similar ways, but further *in vivo* studies are needed to determine the mechanisms of action.

In the present study, progranulin was expressed in the rat myocardium mRNA of the progranulin (GRN) gene was highly upregulated following MI/R injury. Also, after progranulin treatment, its expression was augmented in cardiac tissue, indicating that progranulin may accumulate in cardiac tissue following MI/R injury. Previously published studies have shown that adiponectin expression is upregulated similarly in MI/R injury, and showed that adiponectin accumulated in cardiac tissue from the vascular compartment [32]. However, the cardioprotective effect of endogenous progranulin was not detected in the present study, although it was highly upregulated in cardiac tissue. This finding may be explained by the inability of progranulin to saturate receptors to a level that enhances myocardial contractility, or it may be attributed to a regulatory role for progranulin in cardiac function. Further *in vitro* and *in vivo* studies are required to clarify these roles.

Cardiac myocyte death in MI/R injury is primarily caused by apoptotic activity [33]. The overexpression of Bcl-2 can significantly reduce apoptosis and reduce the size of the myocardial infarct after MI/R injury [34]. Progranulin given before induction of ischemia, increased Bcl-2 expression, indicating that the anti-apoptotic protein may contribute to the cardioprotective effects of progranulin in the setting of MI/R injury. Adiponectin has shown analogous anti-apoptotic characteristics [32], as has CTRP3 [10]. Although progranulin increased the expression of Bcl-2, it did not significantly alter the levels of the apoptotic protein p53. This finding may be explained by the short period of ischemia and reperfusion used in this study, and future studies should include a longer period of reperfusion is to examine the effect of progranulin administration on p53 expression following MI/R injury [35].

Progranulin decreases the production of reactive oxygen species (ROS) from neutrophils by interfering with TNF signaling [16]. Furthermore, it protects against oxidative stress in the brain [30,36], and inhibits NF-κB, a known oxidative stress-responsive transcription factor, which leads to reduced production of ROS by neutrophils [30]. Therefore, the protective role of progranulin in MI/R injury may be, in part, due to its antioxidant activity. However, further research is needed on the antioxidant effects of progranulin. The findings from the present study highlight several characteristics of progranulin that are similar to adipokines that have beneficial effects on the heart.

LY294002, an inhibitor of the PI3-kinase pathway, induces apoptosis in cells by blocking the PI3K/Akt anti-apoptotic pathway. Studies have shown that the PI3K/Akt signaling pathway is involved in protection against MI/R injury [37,38]. Progranulin reduces ischemic neuronal apoptosis [39], and exhibits antioxidant properties in cortical neurons through activation of the PI3K/Akt cell survival pathways [36]. However, to the best of our knowledge, no previous study has investigated whether the PI3K/Akt pathway mediates the cardioprotective effects of progranulin. The findings from this study showed that progranulin exerts its cardioprotective effect through activation of the PI3-K/Akt signaling pathway, which was observed in the preliminary stages of the study [24].

Obesity is associated with ischemic heart disease and its complications, including heart failure [40]. In this study, the administration of progranulin protected against acute myocardial ischemia and improved cardiac function, which may suggest an important physiological and therapeutic function of progranulin in ischemic heart disease. Progranulin may protect against obesity-related cardiovascular complications. However, future clinical studies will be required to determine the role of progranulin in obesity-associated ischemic heart disease.

Conclusions

This study aimed to investigate the effects of progranulin in a rat model of acute myocardial ischemia/reperfusion (MI/R) *in vivo*. The findings demonstrated a protective effect of progranulin in acute MI/R injury in the rat model through the activation of the P13K/Akt pathway. Anti-apoptotic, anti-inflammatory, and inotropic mechanisms may contribute to the cardioprotective effects of progranulin in acute MI/R injury in this model. The mechanisms of the effects of progranulin in the setting of acute MI/R injury require further *in vivo* studies, given the potential clinical role for progranulin in ischemic heart disease.

Conflict of interest

None.

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