Granulocyte colony-stimulating factor reduces the endoplasmic reticulum stress in a rat model of diabetic cardiomyopathy

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Abstract. Prolonged endoplasmic reticulum (ER) stress contributes to the apoptosis of cardiomyocytes, which leads to the development of diabetic cardiomyopathy. Previously, we reported that the granulocyte colony-stimulating factor (G-CSF) reduces the cardiomyocyte apoptosis in diabetic cardiomyopathy; however, the precise mechanisms associated with this process are not yet fully understood. Therefore, in this study, we investigated whether the mechanism of the anti-apoptotic effect of G-CSF was associated with ER stress in a rat model of diabetic cardiomyopathy. Diabetic cardiomyopathy was induced in rats using a high-fat diet combined with the administration of a low-dose of streptozotocin. Diabetic rats were treated with G-CSF or saline for 5 days. Cardiac function was evaluated using serial echocardiography before and 4 weeks after treatment. The rate of cardiomyocyte apoptosis and the expression levels of proteins related to ER stress, including glucose-regulated protein 78 (GRP78), caspase-9, and caspase-12 were analyzed in the cardiac tissue. G-CSF treatment significantly reduced cardiomyocyte apoptosis in the diabetic myocardium and downregulated the expression levels of these proteins in diabetic rats treated with low-dose streptozotocin when compared to that in rats treated with saline. In addition, G-CSF treatment significantly downregulated the expression levels of proteins related to ER stress, such as GRP78, inositol-requiring enzyme-1α (IRE-1α), and C/EBP homologous protein (CHOP) in H9c2 cells under high glucose (HG) conditions. Moreover, G-CSF treatment significantly improved the diastolic dysfunction in serial echocardiography assessments. In conclusion, the anti-apoptotic effect of G-CSF may be associated with the downregulation of ER stress.

Key words: Diabetic cardiomyopathy, Cardiomyocyte, Granulocyte colony-stimulating factor, Endoplasmic reticulum stress

DIABETIC CARDIOMYOPATHY is characterized by diastolic dysfunction, ventricular hypertrophy, and myocardial fibrosis, independent of hypertension and coronary artery disease, which can lead to heart failure in patients with diabetes [1]. Impairment of cardiac insulin metabolic signaling, mitochondrial dysfunction, increased oxidative stress, advanced glycation end products, extracellular matrix stiffness, impaired cardiomyocyte calcium handling, inflammation, activation of the renin–angiotensin–aldosterone system, and prolonged endoplasmic reticulum (ER) stress are factors implicated in the development and progression of diabetic cardiomyopathy [2, 3]. Although the precise mechanisms are not fully understood, overwhelming evidence indicates that cardiomyocyte apoptosis plays an important role in the development of diabetic cardiomyopathy [4, 5]. Cardiac oxidative stress, lipotoxicity, inflammation, and the accumulation of misfolded proteins impair the function of cardiac ER and promote ER stress, inducing an unfolded protein response (UPR) [6]. Together, ER stress and the UPR inhibit cellular protein synthesis and
degradation of misfolded or damaged proteins, ultimately increasing the incidence of cell apoptosis [7]. In addition, cardiomyocyte apoptosis correlates with prolonged ER stress induced by hyperglycemia in experimental settings, suggesting that ER stress is initiated in diabetic hearts and ER stress-induced apoptosis is an important mechanism involved in the pathogenesis and development of diabetic cardiomyopathy [8, 9].

G-CSF has various functions, such as induction of proliferation, survival, and differentiation of hematopoietic cells and mobilization of bone marrow cells [10]. It has been reported that bone marrow cells can differentiate into cardiomyocytes and vascular cells, thereby contributing to myocardium regeneration and angiogenesis in ischemic hearts [11]. Harada et al. discussed the possible molecular mechanisms of G-CSF action in preventing left ventricular remodeling after MI. They found that G-CSF acted directly on a G-CSF receptor expressed on cardiomyocytes and activated the Janus kinase (JAK)–signal transducer of activators of transcription (Jak/Stat) pathway [12]. We previously reported that G-CSF improves cardiac diastolic dysfunction and reduces cardiomyocyte apoptosis through upregulation of the G-CSF receptor in diabetic rats [13, 14]. However, we did not provide further insight into the underlying mechanisms. Therefore, in this study, we explored whether the mechanisms of the anti-apoptotic effects of G-CSF were associated with ER stress in a rat model of diabetic cardiomyopathy.

Materials and Methods

Animals
The experiments were performed in compliance with the ARRIVE guidelines on animal research [15] and the research protocol was approved by the Hanyang University Institutional Animal Care and Use Committee. Male Sprague-Dawley (SD) rats (Koatech, Kyungki-do, Republic of Korea) aged 7 weeks and weighing 210–230 g were used. A rat model of diabetic cardiomyopathy was induced by a high-fat diet (HFD, 60% fat content, D12492; Research Diets, New Brunswick, NJ, USA) and low-dose streptozotocin (STZ, Sigma-Aldrich, St, Louis, MO, USA) [16]. Rats were maintained in a specific pathogen-free facility at the Hanyang University Medical School Animal Experiment Center under conditions of controlled temperature (23 ± 2°C) and humidity (55 ± 5%) with an alternating 12 h light/dark cycle.

Experimental design and drug treatment
The experimental design is shown in Fig. 1. Rats were randomly assigned to one of two dietary regimens: normal chow diet (n = 6) or HFD (n = 16) for an initial period of 7 weeks. After 6 weeks (at age 13 weeks), the HFD group received a single intraperitoneal injection of STZ (30 mg/kg) in 0.1 mM citrate buffer and the normal chow group received an injection of an equivalent volume of citrate buffer vehicle. After one week (at age 14 weeks), fasting blood glucose (FBG) was measured and rats with blood glucose levels ≥200 mg/dL (11.1 mmol/L) were considered to have diabetes mellitus [17]. At 15 weeks of age, diabetic rats were randomly divided into two subgroups for treatment with saline (n = 8) or G-CSF (n = 8). Rats in the G-CSF treatment group were injected intraperitoneally with recombinant human G-CSF (200 μg/kg/day; Leucostim, Dong-A Pharmacological, Seoul, Republic of Korea) for 5 days. Rats in the normal chow group and the diabetic group treated with saline were intraperitoneally injected with an equivalent volume of saline for 5 days. At 18 weeks of age, all the rats were euthanized for laboratory analysis.

Body weight and biochemical analysis
Body weight, FBG, total cholesterol (TC), triglyceride (TG), and fasting insulin levels were measured. Blood samples were collected from tail veins after 8 h of fasting and serum glucose, TC, and TG levels were measured using an Olympus AU400 auto analyzer (Olympus

Fig. 1 Scheme of the animal experiment. Diabetic rats were induced after 7 weeks of feeding with a high-fat diet with low-dose streptozotocin (30 mg/kg) injection and then randomized for treatment for 5 days with G-CSF or with saline administered intraperitoneally. Body weight measurement, biochemical analysis, and echocardiography were performed pre-treatment and post-treatment. At 18 weeks of age, all rats were euthanized for histology and protein analysis. SD rat, Sprague-Dawley rat; G-CSF, granulocyte colony-stimulating factor.
G-CSF reduces the ER stress

GmbH, Hamburg, Germany). Fasting insulin was measured using EZRMI 13 K kits (Millipore, St Charles, Mo, USA) according to the manufacturer’s instructions. Insulin resistance was estimated by homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: HOMA-IR = fasting insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5 [18].

Cell culture

The H9c2 rat cardiomyocyte cell line (ATCC, Manassas, VA, USA) was cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, Grand Island, NY, USA) containing 5.5 mM glucose, 10% fetal bovine serum (FBS; Gibco), and 1% penicillin and streptomycin (Gibco) at 37°C in a humidified incubator with 5% CO₂. At 70% to 80% confluence, the cells were divided into three groups for treatment as follows: DMEM containing 5.5 mM glucose (normal glucose, NG); (2) DMEM containing 33 mM glucose (high glucose; HG); (3) DMEM containing 33 mM glucose (HG) supplemented with G-CSF (500 ng/mL). After 48 h, the cells were harvested for western blot analysis of ER stress-related proteins. All tests were repeated at least three times.

Echocardiography

Echocardiography before and after treatment was performed by anesthetizing rats with an intramuscular injection of a mixture of zoletil 50 (30 mg/kg; Virbac SA, Carros, France) and rompun (10 mg/kg; Bayer Korea, Seoul, Republic of Korea) [19]. The left side of the chest was shaved to obtain clear images. Serial echocardiographic examinations (VIVID E9, GE Healthcare with a 12 probe) were performed by a single sonographer, with rats in the left lateral decubitus position. Left ventricular ejection fraction (LVEF), early peak velocity of the early diastolic filling wave (E), and early mitral annulus velocity during the diastolic phase (E’) were measured [14]. All measurements were used to generate a mean of five consecutive cardiac cycles and the mean values were used in analyses.

TUNEL assay

Apoptotic cells in the myocardium were detected via terminal deoxynucleotidyl transferase (TDT)-mediated dUTP–biotin nick end–labeling (TUNEL) assays of paraffin sections using in situ cell death detection kits (Roche, Mannheim, Germany). Stained sections were photographed using a light microscope (Leica DM 4000 B). Five regions from each digitized image were selected at random and the numbers of healthy and TUNEL-positive (apoptotic) nuclei were quantified. The apoptotic index was calculated as the number of TUNEL-positive nuclei/total number of nuclei [13]. All data were evaluated by an independent, blinded investigator.

Western blotting

Heart halves were homogenized and total protein was extracted using protein lysis buffer (Pro-prep; iNtRON, Seongnam, Republic of Korea). Cardiac tissue samples containing 60 μg total proteins were boiled for 20 min and loaded onto sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gels (8% stacking and 10% or 15% separating gels). Separated proteins were transferred to nitrocellulose membranes (0.45 μm pore size; Bio-Rad, Hercules, CA, USA) or Immobilon-P transfer poly (vinylidene fluoride) (PVDF) membrane (0.45 μm pore size; Millipore, Billerica, MD, USA). After blocking in 5% bovine serum albumin solution (Sigma-Aldrich) or 5% skim milk solution (BD Biosciences, San Diego, CA, USA) for 60 min, the membranes were incubated with primary antibody overnight at 4°C. The primary antibodies used are shown in Table S1. Blots were incubated with horse radish peroxidase (HRP)-conjugated anti-rabbit antibody (1:2,000; Jackson Immunoresearch, West Grove, Iowa, USA) or antimouse antibody (1:2,000; Jackson Immunoresearch) for 1 h at room temperature. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control. Positive protein bands were visualized using an enhanced chemiluminescence (ECL) kit (GenDEPOT, Barker, NY, USA) and the results were quantified using an image analyzer (Image lab 3.0, Bio-Rad).

Statistical analyses

The Statistical Package for the Social Sciences (SPSS) software (version 22.0; SPSS Inc., Chicago, IL, USA) was used for statistical analyses. All data are expressed as the mean ± standard deviation (SD), except for histological and echocardiographic data, which are expressed as the mean ± standard error (SE). Data were analyzed using one-way analysis of variance (ANOVA) test for multiple comparisons, and post hoc multiple comparisons were made with Tukey’s test (equal variances assumed) or Dunnett T3 test (equal variances not assumed). Statistical significance was set at p < 0.05.

Results

Body weight and biochemical analysis

At the end of the experiments, there were no significant differences in body weight between the diabetic and normal rats. Diabetic rats showed significantly increased FBG, TC, and TG levels compared to those in normal rats. Diabetic rats treated with saline also showed significantly increased HOMA-IR levels compared with those in normal rats but the differences in HOMA-IR levels
were not significant between diabetic rats treated with G-CSF and normal rats (Table 1). These results confirmed the successful development of a diabetic rat model using a combination of HFD and low-dose STZ.

**Effect of G-CSF on cardiac diastolic dysfunction**

Echocardiography was performed before and after treatment for cardiac function. Before treatment, LVEF was maintained, but the E’ velocity was significantly lower and the E/E’ ratio was significantly higher in diabetic rats than in normal rats, suggesting that diabetic rats exhibited diastolic dysfunction. After treatment, echocardiography revealed that the E’ velocity was significantly higher and the E/E’ ratio was significantly lower in diabetic rats treated with G-CSF than in diabetic rats treated with saline. LVEF and E velocity did not differ significantly between groups. A significant increase in E’ velocity (2.53 ± 0.51 cm/s vs. 4.00 ± 0.55 cm/s, p < 0.05) and decrease in E/E’ ratio (27.35 ± 5.01 vs. 17.19 ± 2.13, p < 0.05) was detected in diabetic rats treated with G-CSF compared with the measurements taken before treatment (Table 2 and Fig. S1). These results showed that G-CSF treatment improved cardiac diastolic dysfunction in a rat model of diabetic cardiomyopathy.

**Effect of G-CSF on cardiomyocyte apoptosis in cardiac tissue**

The apoptotic index was significantly higher in diabetic rats treated with saline than in normal control rats. Diabetic rats treated with G-CSF significantly normalized apoptotic index compared to diabetic rats treated with saline (25.12 ± 4.24% vs. 34.51 ± 3.93, p < 0.05) (Fig. 2A and B). Bcl-2 protein levels were significantly higher in diabetic rats treated with G-CSF than in diabetic rats treated with saline (82.86 ± 14.76% vs. 52.99% ± 19.58%, p < 0.05) (Fig. 2C and D). These results showed that G-CSF treatment reduced cardiomyocyte apoptosis in diabetic myocardium.

**Effect of G-CSF on ER stress in cardiac tissue**

To understand the molecular basis of the anti-apoptotic effects of G-CSF in diabetic rat hearts, we examined the expression of GRP78, caspase-9, and caspase-12, which are markers of ER stress. Western blotting analysis showed that GRP78 expression was significantly lower in diabetic rats treated with G-CSF than in diabetic rats treated with saline (82.86 ± 14.76% vs. 52.99% ± 19.58%, p < 0.05) (Fig. 2C and D). These results showed that G-CSF treatment reduced cardiomyocyte apoptosis in diabetic myocardium.

### Table 1 Body weight and biochemical analysis

|                  | Normal     | Saline     | G-CSF      |
|------------------|------------|------------|------------|
| BW (g)           | 400.80 ± 18.81 | 383.88 ± 38.01 | 383.38 ± 28.28 |
| FBG (mg/dL)      | 130.20 ± 1.50 | 258.60 ± 56.10* | 239.00 ± 43.91* |
| TC (mg/dL)       | 95.00 ± 0.50  | 113.83 ± 6.33* | 116.75 ± 6.20*  |
| TG (mg/dL)       | 30.00 ± 3.19  | 51.83 ± 3.82*  | 45.33 ± 6.53*   |
| HOMA-IR          | 1.65 ± 0.28   | 3.03 ± 0.75*   | 2.85 ± 0.73     |

BW, body weight; FBG, fasting blood glucose; TC, total cholesterol; TG, triglyceride; HOMA-IR, homeostasis model assessment of insulin resistance = fasting insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5; Normal, normal rats; Saline, diabetic rats treated with saline; G-CSF, diabetic rats treated with granulocyte-colony stimulating factor. All data are expressed as the mean ± SD. * p < 0.05 vs. normal group (n = 6–8 per group).

### Table 2 Echocardiographic data before and after treatment

|                  | Before treatment | After treatment |
|------------------|------------------|-----------------|
|                  | Normal   | Saline   | G-CSF    | Normal   | Saline   | G-CSF    |
| EF (%)           | 65.59 ± 1.17 | 65.33 ± 1.41 | 67.88 ± 1.13 | 68.49 ± 1.87 | 66.84 ± 0.75 | 67.09 ± 0.94 |
| E (cm/sec)       | 70.27 ± 11.08 | 68.24 ± 8.56 | 67.58 ± 5.13 | 73.00 ± 9.66 | 67.00 ± 6.57 | 67.66 ± 6.61 |
| E’ (cm/sec)      | 4.72 ± 0.75  | 2.67 ± 0.45* | 2.53 ± 0.51* | 4.50 ± 0.53  | 2.55 ± 0.20* | 4.00 ± 0.55† |
| E/E’             | 13.73 ± 1.12 | 24.47 ± 5.18* | 27.35 ± 5.01* | 16.10 ± 2.85 | 27.60 ± 3.56* | 17.19 ± 2.13† |

EF, ejection fraction; E, peak velocity of the early diastolic filling wave; E’, early mitral annulus velocity during the diastolic phase; Normal, normal rats; Saline, diabetic rats treated with saline; G-CSF, diabetic rats treated with granulocyte-colony stimulating factor. All data are expressed as the mean ± SE. * p < 0.05 vs. normal group, † p < 0.05 vs. saline group (n = 6–8 per group).
(91.40 ± 21.91% vs. 139.23 ± 17.66% for caspase-9; 46.00 ± 19.19% vs. 123.65 ± 32.31% for caspase-12; all p < 0.05) (Fig. 3). These results showed that the anti-apoptotic effect of G-CSF was significantly associated with the downregulation of ER stress in the diabetic myocardium of rats.

**Effect of G-CSF on ER stress in H9c2 cells**

To clarify the effect of G-CSF on ER stress, we measured the protein levels of GRP78, IRE-1α, and CHOP. G-CSF treatment reduced the expression levels of GRP78, IRE-1α, and CHOP proteins in H9c2 cell under HG conditions compared to those observed in non-treated H9c2 cells under HG conditions (39.5 ± 8.09% vs. 60.9 ± 18.46% for GRP78, 61.21 ± 10.21% vs. 92.15 ± 5.66% for IRE-1α; 24 ± 9.39% vs. 68.65 ± 10.31% for CHOP; all p < 0.05) (Fig. 4). Taken together, these results also suggest that the anti-apoptotic effect of G-CSF in H9c2 cardiac cells under diabetic conditions is significantly linked to the downregulation of ER stress.

**Discussion**

In this study, we showed that G-CSF promoted the cardiac diastolic dysfunction and reduced cardiomyocyte apoptosis in a rat model of diabetic cardiomyopathy. Moreover, G-CSF downregulated GRP78, caspase-9, caspase-12, IRE-1α, and CHOP, suggesting a molecular explanation that the anti-apoptotic effects of G-CSF in the rat model of diabetic cardiomyopathy might be mediated by the downregulation of ER stress.

Diabetic cardiomyopathy is characterized in its early stages by diastolic dysfunction and later by clinical heart failure in the absence of hypertension and coronary heart disease.
disease [19]. Systemic and insulin resistance, activation of the renin-angiotensin-aldosterone system, and increase in oxidative stress, inflammation, and ER stress are pathophysiological factors associated with diabetes. All promote cardiac dysfunction and induce cardiomyocyte apoptosis in the diabetic myocardium [20-22]. In addition, high-glucose conditions also increase cardiomyocyte apoptosis through an ER stress-induced apoptosis pathway in vitro [23].

The ER plays an integral role in lipid synthesis, Ca\(^{2+}\) handling, and protein folding and modification [24]. Interruption of these processes by stimuli, including hypoxia, hyperglycemia, and oxidative stress, can lead to the accumulation of unfolded proteins, resulting in an evolutionarily conserved cell stress response, termed the UPR or ER stress [25]. Lakshmanan et al. reported that ER stress contributes to diabetic cardiomyopathy in transgenic non-obese type 2 diabetic rats [26]. In addition, severe and prolonged ER stress extensively impairs ER functions and activates the apoptotic pathway in diabetic cardiomyopathy [27], which is known to be an important mechanism in the development of diabetic cardiomyopathy [28-30].

The major finding of this study was that the G-CSF showed anti-apoptotic effect via downregulation of ER stress in the diabetic myocardium and H9c2 cells under HG conditions. ER stress is mediated by three ER transmembrane receptors: pancreatic ER kinase-like ER kinase (PERK), activating transcription factor 6 (ATF-6), and inositol-requiring enzyme-1α (IRE1α). All three ER stress receptors are maintained in an inactive state through their association with the ER chaperone GRP78 [31]. Therefore, GRP78 induction is an indicator of ER stress and GRP78 is used to monitor ER stress [32]. However, severe and prolonged ER stress switches signaling from pro-survival to pro-apoptotic [33]. Caspase-12 is specifically localized on the cytoplasmic side of the ER and acts as the major local regulatory factor for apoptosis [34]. Caspase-12 is also activated by ER stress, resulting in a caspase-9 activation cascade that induces cell apoptosis [35]. Li et al. provided evidence that increase in GRP78 levels, activation of caspase-12 in diabetic myocardium, and ER stress-induced cardiomyocyte apoptosis are responsible for diabetic heart failure [36]. Xiong et al. reported that melatonin ameliorated myocardial apoptosis by suppressing ER stress in rats.

Fig. 3 Effect of G-CSF on ER stress in cardiac tissue. (A) Representative images of expression of autophagy-related proteins GRP78, caspase-9, and caspase-12 in diabetic myocardium by western blotting at 18 weeks of age (4 weeks after G-CSF or saline treatment). GAPDH was the loading control. (B)–(D) Western blotting quantitation for GRP78, caspase-9, and caspase-12. Proteins are normalized to GAPDH. Normal, normal rats; Saline, diabetic rats treated with saline; G-CSF, diabetic rats treated with G-CSF; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G-CSF, granulocyte colony-stimulating factor. All data are expressed as the mean ± standard deviation (SD). * \(p < 0.05\) vs. normal group, † \(p < 0.05\) vs. saline group (\(n = 6–8\) per group).
with diabetic cardiomyopathy [37]. In addition, Liu et al. and Wu et al. reported that reduction of ER stress reduces cardiomyocyte apoptosis in diabetic rats [38, 39]. The mechanisms regulating the expression of Bcl-2 family proteins during ER stress-induced apoptosis are now understood [7]. Distelhorst et al. suggested that overexpression of the anti-apoptotic protein Bcl-2 protects cells from ER stress-induced apoptosis of lymphoma cells [40]. Menzie-Suderam et al. demonstrated that G-CSF protects against ER stress-induced apoptosis of neurons and upregulates the anti-apoptotic protein Bcl-2 in a rat model of ischemic stroke [41]. In addition, G-CSF has been reported to reduce neutrophil apoptosis by downregulating caspase-9 and caspase-12 [42]. Thandavarayan et al. suggested that GRP78 and IRE-1α protein levels were significantly increased in the diabetic heart compared to the wild-type control. Moreover, cardiac apoptosis and the expression levels of CHOP, caspase-12, and cleaved caspase-12 proteins were significantly increased in the diabetic heart. ER stress-induced apoptosis pathways, at least in part, include the regulation of CHOP and caspase-12 via the IRE-1α pathway [43]. In this study, we showed that G-CSF upregulated Bcl-2 and downregulated cardiac ER stress, as indicated by downregulation of GRP78, caspase-9, and caspase-12 in the diabetic myocardium and GRP78, IRE-1α, and CHOP in H9c2 cells under HG conditions. Thus, we propose that down-regulation of ER stress by G-CSF treatment could be a viable therapeutic strategy to reduce cardiomyocyte apoptosis in diabetic cardiomyopathy.

This study has several limitations. First, we provide only a new mechanistic understanding of the anti-apoptosis and ER stress reduction caused by G-CSF, and not the causal relationships between apoptosis and ER stress. Further studies are needed to confirm whether improvement in cardiomyocyte apoptosis is mediated through the ER stress-induced apoptosis pathway. Second, we confirmed that only GRP78, caspase-9, caspase-12, IRE-1α, and CHOP were associated with the ER stress-induced apoptosis pathway. We did not confirm other sensors of ER stress, such as PERK, ATF-6, and downstream signaling molecules for the IRE-1α pathway (e.g., X-box binding protein 1 [XBP1], apoptosis signal-regulating kinase 1 [ASK1], and JNK [cJUN NH2-terminal kinase]). Further investigation on such ER stress sensors and downstream signaling molecules is needed. Third, we showed changes in IRE-1α and CHOP expression only in H9c2 cells and did not verify the
changes in the diabetic myocardium. Finally, we could not directly investigate the action of G-CSF on cardiomyocytes through the G-CSF receptor or through mobilization of bone marrow stem cells. Further studies on the precise mechanism involved in the anti-apoptotic effect of G-CSF are worth exploring.

In conclusion, our study indicates that G-CSF improves the cardiac function and reduces cardiomyocyte apoptosis in diabetic cardiomyopathy. G-CSF downregulated GRP78, caspase-9, caspase-12, IRE-1α, and CHOP, which are important components of the ER stress-induced apoptosis pathway. Our data suggest that the beneficial effects of G-CSF may be related to the downregulation of ER stress, providing new insights into the potential molecular mechanism of cardiomyocyte apoptosis.

Acknowledgments

Not applicable.

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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