

Original Paper

Polyamine Depletion Attenuates Isoproterenol-Induced Hypertrophy and Endoplasmic Reticulum Stress in Cardiomyocytes

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Key Words

Cardiac hypertrophy • Apoptosis • Polyamine • Endoplasmic reticulum stress

Abstract

Background/Aim: Polyamines (putrescine, spermidine and spermine) play an essential role in cell growth, differentiation and apoptosis. Hypertrophy is accompanied by an increase in polyamine synthesis and endoplasmic reticulum stress (ERS) in cardiomyocytes. The present study was undertaken to elucidate the molecular interactions between polyamines, ERS and cardiac hypertrophy. **Methods:** Myocardial hypertrophy was simulated by incubating cultured neonatal rat cardiomyocytes in 100 nM isoproterenol (ISO). Polyamine depletion was achieved using 0.5 mM difluoromethylornithine (DFMO). Hypertrophy was estimated using cell surface area measurements, total protein concentrations and atrial natriuretic peptide (ANP) gene expression. Apoptosis was measured using flow cytometry and transmission electron microscopy. Expression of ornithine decarboxylase (ODC) and spermidine/spermine N1-acetyltransferase (SSAT) were analyzed via real-time PCR and Western blotting. Protein expression of ERS and apoptosis factors were analyzed using Western blotting. **Results:** DFMO (0.5 mM and 2 mM) treatments significantly attenuated hypertrophy and apoptosis induced by ISO in cardiomyocytes. DFMO also decreased lactate dehydrogenase (LDH) and malondialdehyde (MDA) level in the culture medium. In addition, DFMO (0.5 mM) down regulated the expression of ODC, glucose-regulated protein 78 (GRP78), C/EBP homologous protein (CHOP), cleaved caspase-12, and Bax and up regulated the expression of SSAT and Bcl-2. Finally, these changes were partly reversed by the addition of exogenous putrescine (0.5 mM). **Conclusion:** The data presented here suggest that polyamine depletion could inhibit cardiac hypertrophy and apoptosis, which is closely related to the ERS pathway.

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Introduction

Polyamines (spermine, spermidine and putrescine) are polycationic aliphatic amines that influence nucleic acid structure and stability, modulate ion channel activity, govern mRNA translation rates, and are important mediators of cellular growth and division. In cardiomyocytes, increasing evidence indicates that polyamines play a role in various aspects of cardiac disease [1, 2]. Ornithine decarboxylase (ODC) is the enzyme that decarboxylates ornithine to the diamine putrescine, the precursor of spermidine and spermine and the rate-limiting step in polyamine biosynthesis in mammalian cells [3]. Inhibition of ODC, using the ornithine analog difluoromethylornithine (DFMO), has proved an effective means of depleting cellular polyamines in mammals and is clinically approved for humans.

The endoplasmic reticulum (ER) is an organelle that is responsible for regulating protein synthesis, protein folding, trafficking and intracellular calcium levels [4, 5]. Accumulation of misfolded or unfolded proteins in the ER can result in endoplasmic reticulum stress (ERS) [6]. In the early stages of ERS, a series of signaling pathways assist in protein folding, severe or prolonged ERS will trigger cellular apoptosis. C/EBP homologous protein (CHOP) is an important transcription factor that mediates protein kinase R-like ER kinase (PERK) activation-induced apoptosis in ERS [7]. Caspase-12 mediated apoptosis is a specific apoptotic pathway in the ER, which is independent on mitochondria or death receptor activation. ERS is required for proper cardiac development, and it provides certain protective mechanisms against cardiac damage caused by various stresses, as well as being involved in cardiovascular pathological processes, such as myocardial infarction, ischemia/reperfusion and pressure overload-induced hypertrophy [8-10].

Cardiac hypertrophy, an enlargement of the heart, is a major risk factor that is associated with adverse cardiovascular outcomes [11]. Although our previous studies demonstrated that DFMO can attenuate cardiomyocyte hypertrophy by inhibiting the NO/cGMP-dependent protein kinase-1 pathway, no study, to date, has directly addressed the effect of DFMO on ERS and the underlying mechanisms in cardiac hypertrophy [12, 13]. We hypothesize that the protective effects of DFMO on cardiomyocytes may be at least partially due to ERS.

Materials and Methods

Materials

Isoproterenol (ISO), DFMO, putrescine (Pu) and trypsinase were purchased from Sigma-Aldrich (St. Louis, MO, USA). ODC, spermidine/spermine N1-acetyltransferase (SSAT), glucose-regulated protein 78 (GRP78), CHOP, caspase-12, Bax and Bcl-2 primary antibodies were obtained from Santa Cruz Biotechnology Incorporated (Santa Cruz, CA, USA). Trizol reagent and the PrimerScript RT reagent kit were purchased from TakaRa Biotechnology Incorporated (TakaRa Bio Inc, Japan). The Western blot kit and β -actin antibody were purchased from Boster (Wuhan, China). High glucose Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco BRL (Gaithersburg, MD, USA). Assay kits for malondialdehyde (MDA) and lactate dehydrogenase (LDH) were purchased from Jiancheng Bioengineering Institute (Nanjing, China).

Cardiomyocytes culture and treatment

Primary cultures of neonatal rat cardiomyocytes were established and maintained as previously described [14]. Neonatal rat cardiomyocytes were prepared from 2- to 3-day-old neonatal Wistar rats. The rats were handled in accordance with the Guide for the Care and Use of Laboratory Animals, published by the China National Institutes of Health. Briefly, the heart were minced and dissociated with 0.25% trypsinase. Dispersed cells were seeded at 2×10^5 cells/cm², in 60-mm culture dishes, with DMEM supplemented with 10% fetal bovine serum (FBS), and then cultured in a 5% CO₂ incubator at 37°C.

Treatment protocol

Three days after the cells were seeded, the cardiomyocytes were randomly divided into the following four groups: (1) control group: cardiomyocytes were continuously cultured for 72 h in DMEM; (2) ISO

group: cardiomyocytes were treated with 100 nM ISO for 48 h; (3) pre-DFMO group: cardiomyocytes were pre-incubated with 0.1 mM, 0.5 mM or 2 mM DFMO for 24 h, and then treated with 100 nM ISO for 48 h; (4) pre-(DFMO+Pu) group: cardiomyocytes were pre-incubated with 0.5 mM DFMO and 0.5 mM Pu for 24 h, and then treated with 100 nM ISO for 48 h. All drugs were dissolved in the pre-warmed culture medium and added directly to the cardiomyocytes. In control groups, equivalent volumes of medium were added.

Measurement of the surface area and total protein concentrations of cardiomyocytes

Cardiomyocytes were counted from a minimum of three dishes per group using phase-contrast microscopy. The cellular surface area was measured by the Image Analysis System (Olympus). Ten fields were randomly chosen for each group, and 10 cardiomyocytes randomly chosen for each field. The total protein concentrations was measured by Bradford's method in each group.

Assessment of apoptosis

Apoptosis was evaluated using double fluorescence staining with annexin V/propidiumiodide (PI). Cardiomyocytes (1×10^5 cells per well) were pre-treated with DFMO and DFMO+Pu for 24 h, and then incubated with ISO (100 nM) for 48 h. After treatment, cardiomyocytes were washed three times with ice-cold PBS, and then treated with 5 mL PI and 10 mL annexin V-FITC (Jingmei Biotech, Shanghai, China) at room temperature for 10 min in the dark. Apoptosis of cardiomyocytes was examined by flow cytometer (Becton, Dickinson and Company, USA) within 1 h.

Transmission electron microscopy

Cardiomyocytes were harvested and fixed with 3.0% glutaraldehyde and 1.5% paraldehyde, washed in phosphate buffer saline (PBS), and fixed in osmium tetroxide. Cells were then dehydrated in an ethanol series, embedded in epoxy resin and examined under a transmission electron microscope (Hitachi, TEM-HT7700).

Real time-PCR analysis of ANP, ODC and SSAT

Total RNA was extracted from the cardiomyocytes using the phenol guanidine isothiocyanate method (Trizol kit, TaKaRa) as per the manufacturer's instructions. The RNA was reverse transcribed with oligo-dT and Superscript First-Strand Synthesis System for RT-PCR (TaKaRa), according to the manufacturer's instructions. The cDNA was then amplified using an iCycler (ABI) and the Brilliant SYBR Green QPCR master mix (TaKaRa). Relative quantification of gene expression was normalized using β -actin as the control gene. The nucleotide sequences of the primers used are: (1) ANP: sense 5-GGGAAGTCAACCGTCTCA-3, antisense 5-GGGCTCCAATCCTGTCAAT-3; (2) ODC: sense 5-GCTTTCTATGTTGCGGACCT-3, antisense 5-TGCTCACTATGGCTCTGCTG-3, (3) SSAT: sense 5-GTGAGCATCCATCCAAAGC-3, antisense 5-ATTCTGCCTCCAAACCACAT-3.

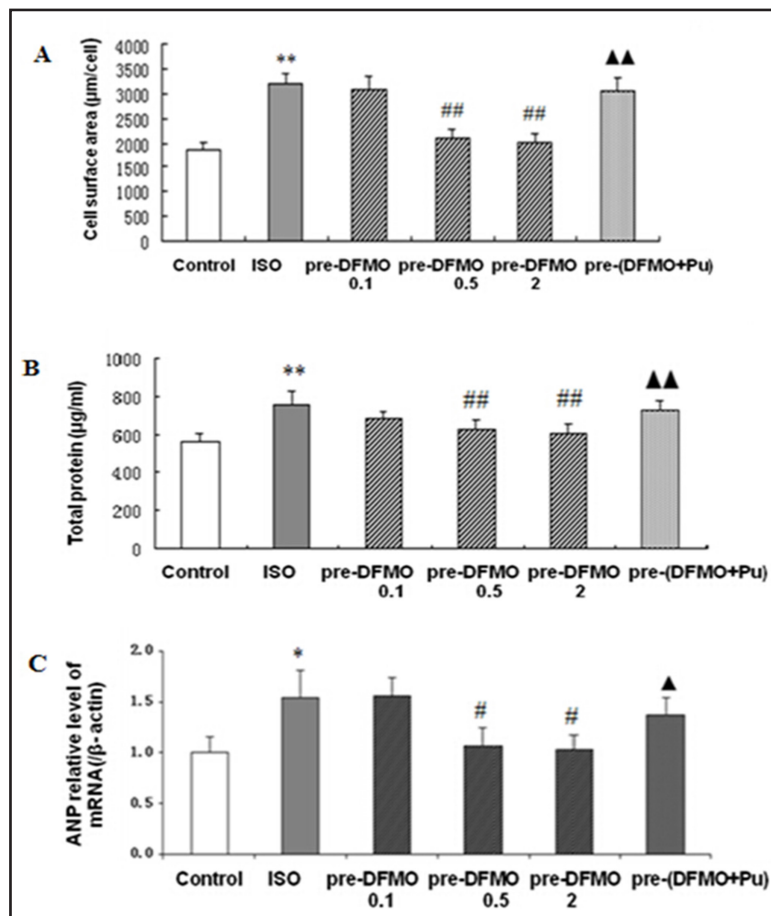
Western blotting analysis of ODC, SSAT, GRP78, CHOP, caspase-12, Bcl-2, and Bax

Total proteins were prepared from neonatal rat cardiomyocytes as described previously [15]. Total protein samples (30 μ g) were loaded in to 10% sodium dodecyl sulfate-polyacrylamide gels, electrophoresed, and then transferred to a polyvinylidene fluoride membrane. Membranes were blocked with 5% skimmed milk in Tris-buffered saline, with 0.1% Tween 20 (TBST) for 1 h at room temperature, and then incubated overnight at 4°C with primary antibody ODC (1:500), SSAT (1:500), GRP78 (1:1000), CHOP (1:500), caspase-12 (1:1000), Bcl-2 (1:500) and Bax (1:500). The membrane was next washed three times in 1× TBST, and incubated with anti-IgG antibody conjugated with peroxidase (1:5000) in TBST for 1 h at room temperature. Using an enhanced chemiluminescence detection kit (Pierce Chemical Company, Rockford, IL, USA). β -actin expression was used as the control.

Measurement of LDH activity and MDA content

The level of MDA in the culture medium was measured using a commercial thiobarbituric acid-reactive substances assay kit (Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's instructions. MDA values were expressed as nmole per gram of protein. The activity of lactate dehydrogenase (LDH) in the culture medium, as an indicator of cytotoxicity, was measured spectrophotometrically using a commercially available assay kit (Jiancheng Bioengineering Institute, Nanjing, China).

Fig. 1. Hypertrophic state in neonatal rat cardiomyocytes. Cardiomyocytes were pretreated with DFMO (0.1 mM, 0.5 mM and 2 mM) or 0.5 mM (DFMO + Pu) for 24 h, and then treated with 100 nM ISO. Data are means \pm S.E.M. of four determinations. * P < 0.05 or ** P < 0.01 versus control group; # P < 0.05 or ### P < 0.01 versus ISO group; Δ P < 0.05 or $\Delta\Delta$ P < 0.01 versus pre-DFMO (0.5 mM) group. (A) The surface area of cardiomyocytes. (B) The total protein concentrations of cardiomyocytes. (C) The gene expression of ANP determined by real-time PCR.



Statistical analysis

Data from at least three independent experiments for each condition were collected. Values are means \pm S.E.M. Comparisons among the groups were carried out using Kruskal-Wallis one-way ANOVA with P < 0.05 considered to be statistically significant.

Results

Hypertrophic occurrences in neonatal rat cardiomyocytes

To investigate the effect of DFMO on hypertrophy, cardiomyocytes were treated with ISO, DFMO and putrescine. Cardiomyocyte hypertrophy was evaluated by the following; namely surface area of cardiomyocytes, total protein concentrations and mRNA expression of ANP (a cardiac hypertrophy marker gene). The results showed ISO increased surface area (P < 0.01), total protein concentrations (P < 0.01), and up regulated mRNA expression of ANP (P < 0.05) compared with the control group. In contrast, after DFMO treatment (0.5 mM and 2.0 mM), hypertrophy markers were significantly decreased (P < 0.01 or P < 0.05 versus ISO group). Pretreatment with 0.1 mM DFMO had no effect on hypertrophy. In the pretreated 0.5 mM DFMO and 0.5 mM Pu group, hypertrophy increased relative to the DFMO treatment (P < 0.01 or P < 0.05 versus DFMO group) (Fig. 1 A- C).

DFMO inhibited ISO-induced apoptosis of cardiomyocytes

In apoptotic cells, the membrane phosphatidylserine translocates from the inner to the outer surface of the plasma membrane, while the membrane remains physically intact. Apoptotic cells were stained with annexin V-fluorescein isothiocyanate (FITC), which binds

Fig. 2. Effect of DFMO on cardiomyocytes apoptosis induced by ISO. Cardiomyocytes were pretreated with 0.5 mM DFMO or 0.5 mM (DFMO + Pu) for 24 h, and then treated with 100 nM ISO. Apoptosis was measured by annexin V/PI staining (flow cytometric analysis). * $P < 0.05$ versus control group; # $P < 0.05$ versus ISO group; ▲ $P < 0.05$ versus pre-DFMO group.

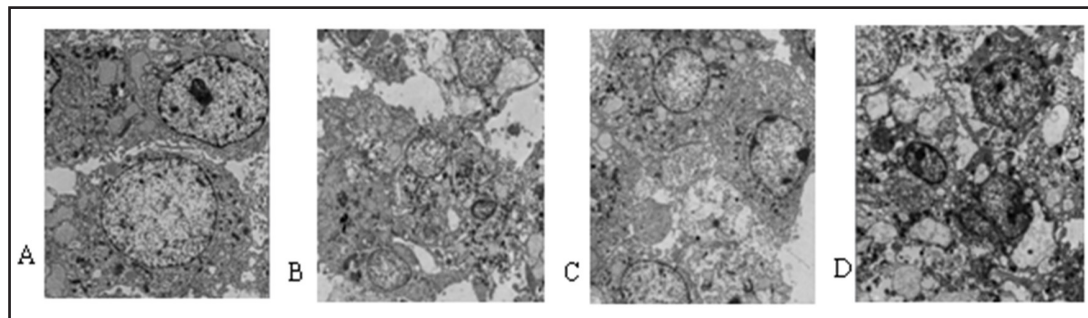
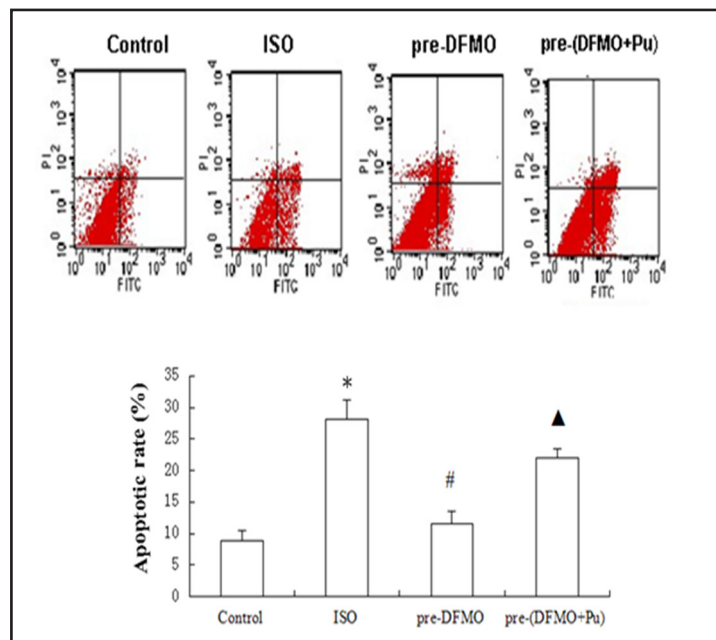


Fig. 3. Ultrastructural changes in cardiomyocytes. (A) Control group (B) ISO group (C) 0.5 mM pre-DFMO group (D) 0.5 mM pre-(DFMO + Pu) group (magnification $\times 8000$). In the ISO and pre-(DFMO + Pu) group, nuclear chromatin margination, aggregation, and condensation and mitochondrial swelling and vacuolisation were observed. There was no significant change in nuclear chromatin in the control group. Cellular injury was lessened in the pre-DFMO group when compared with the ISO group.

phosphatidylserine with high affinity, resulting in green fluorescence when excited at 620 nm. Necrotic cells lose the physical integrity of their plasma membrane and are able to be stained with both annexin V-FITC and PI. Living cells remain unstained. Flow cytometry analysis detected minimal apoptosis in the control group, the basal early apoptotic rate was 8.92%. Apoptosis significantly increased to 28.05% when cells were exposed to ISO ($P < 0.05$ versus control group). The 0.5 mM DFMO treatment group demonstrated decreased apoptosis (11.64%) compared to the ISO group ($P < 0.05$ versus ISO group). In contrast, the 0.5 mM DFMO and 0.5 mM Pu treatment group showed an increase in apoptosis relative to the DFMO treatment group (21.93%) ($P < 0.05$ versus pre-DFMO group) (Fig. 2A and 2B).

The effect of DFMO on morphological changes of cardiomyocytes

The results of transmission electron microscopy showed that the nuclear membrane was intact, even distribution of nuclear chromatin, and intact mitochondria structures in the control group. Morphological changes characteristic of apoptosis, including nuclear chromatin margination, aggregation, and condensation, as well as swelling and vacuolisation of mitochondria were observed in the ISO and pre-(DFMO+Pu) treatment groups. Compared

Fig. 4. Effect of DFMO on ODC and SSAT gene and protein levels in cardiomyocytes. (A) ODC and (B) SSAT protein levels as shown by Western blot. The intensity of each band was quantified by densitometry, and data were normalized to β -actin. (C) ODC and SSAT gene expression as determined by real-time PCR. Control group expression levels were considered the basal levels, and data from other experimental groups are expressed as fold change from the control group. The fold change values represent the mean \pm S.E.M. of four determinations. * $P < 0.05$ or ** $P < 0.01$ versus control group. # $P < 0.05$ or ## $P < 0.01$ versus ISO group. $\Delta P < 0.05$ or $\Delta\Delta P < 0.01$ versus 0.5 mM pre-DFMO group.

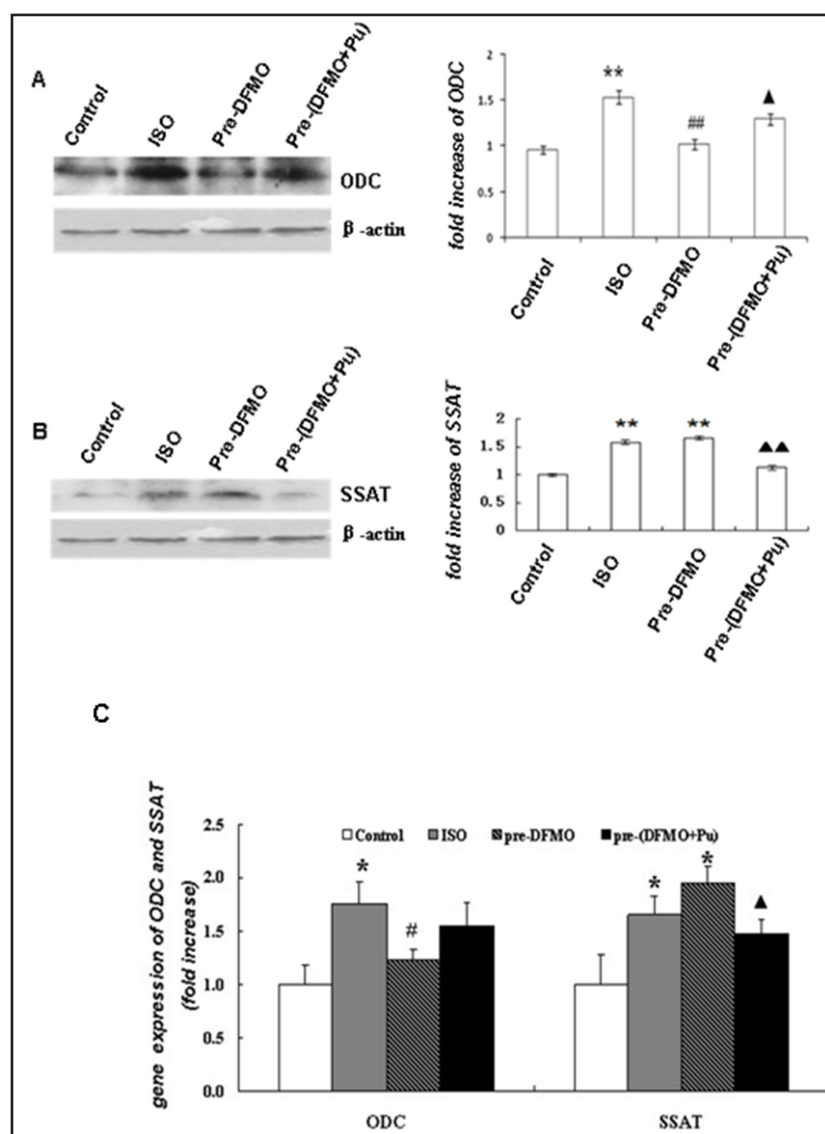


Table 1. LDH and MDA levels in culture medium. Data are means \pm S.E.M. of four separate experiments. ^a $P < 0.01$ versus control group, ^b $P < 0.05$ versus ISO group

Group	LDH (U/mL)	MDA (nmol/mL)
Control	21.8 \pm 2.4	7.6 \pm 0.9
ISO	40.2 \pm 2.6 ^a	15.8 \pm 1.4 ^a
Pre-DFMO (0.5 mM)	33.5 \pm 1.8 ^b	11.5 \pm 1.3 ^b
Pre-(DFMO+Pu) (0.5 mM)	41.8 \pm 2.3	16.1 \pm 1.1

to the ISO group, these morphological changes were less severe in the 0.5 mM DFMO-treated group (Fig. 3A-D).

DFMO inhibited ISO-induced LDH and MDA levels

We measured the level of LDH and MDA of the culture medium to determine whether DFMO protects cardiomyocytes. Compared with the control group, the levels of LDH and MDA were significantly increased in the ISO group ($P < 0.01$). In the DFMO-pretreated group, LDH and MDA levels were significantly decreased ($P < 0.05$ versus the ISO group).

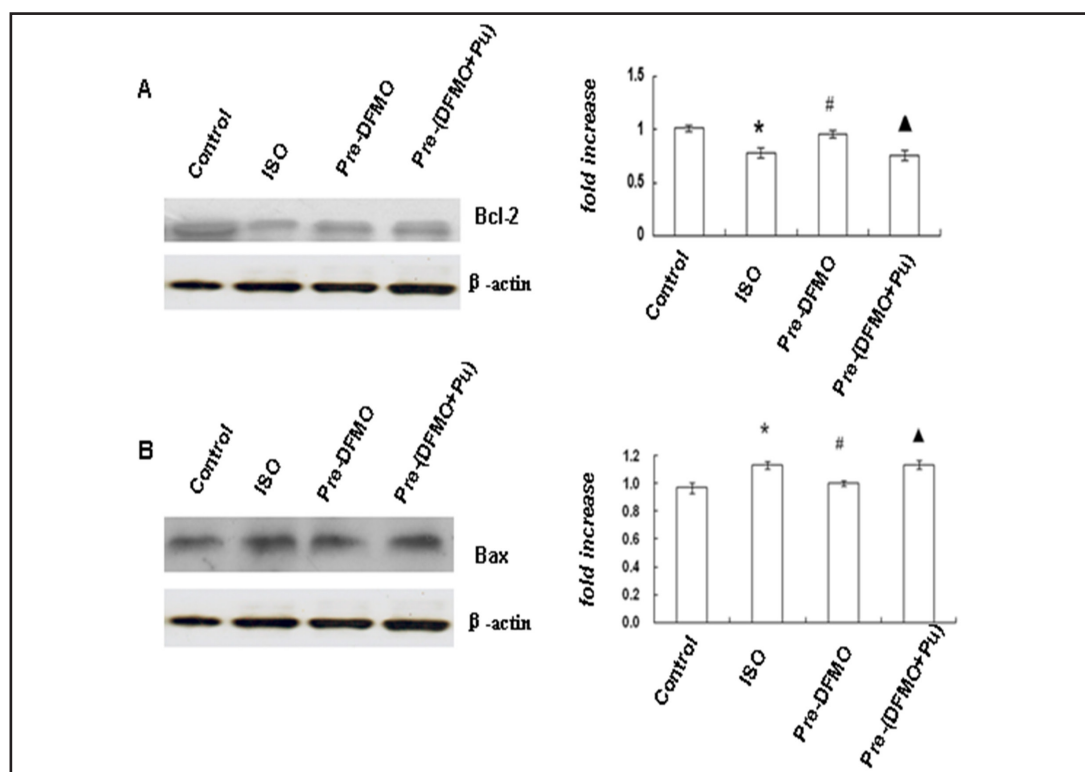


Fig. 5. DFMO reverses Bcl-2 and Bax expression patterns induced by ISO in neonatal rat cardiomyocytes. Bcl-2 (A) and Bax (B) protein levels were detected by Western blot. The control group's protein levels were considered basal levels, and data from the other experimental groups are expressed as fold change from the control group. The fold change values represent the mean \pm S.E.M. of four determinations. * $P < 0.05$ versus control group. # $P < 0.05$ versus ISO group. $\Delta P < 0.05$ versus 0.5 mM pre-DFMO group.

No changes of LDH and MDA were observed in pre-(DFMO+Pu) group compared with the ISO group (Table 1).

The effect of DFMO on the gene and protein expression of ODC and SSAT

The gene expression of ODC and SSAT were increased significantly in the ISO group compared to the control group ($P < 0.05$), the gene expression of ODC was decreased by DFMO pretreatment. However, the gene expression of SSAT was increased in pre-DFMO group and pre-(DFMO + Pu) group. The protein expression of ODC and SSAT was increased in the ISO group ($P < 0.01$) and DFMO decreased the protein expression of ODC ($P < 0.01$). These changes were reversed by the addition of exogenous putrescine ($P < 0.05$ or $P < 0.01$ versus pre-DFMO group) (Fig. 4A-C).

The effect of DFMO on the protein expression of Bcl-2 and Bax

Bcl-2 is an anti-apoptotic factor, responsible for shifting the balance away from cell survival and toward cell apoptosis, and is involved in multiple forms of heart disease. The protein expression of Bcl-2 was decreased and the protein expression of Bax was increased in ISO group compared to the control group. Pretreatment with 0.5 mM DFMO up regulated the expression of Bcl-2 and down regulated the expression of Bax when compared with the ISO group. Compared with the DFMO group, pretreatment with 0.5 mM DFMO and 0.5 mM Pu reversed the expression patterns of Bcl-2 and Bax (Fig. 5A and 5B, all $P < 0.05$).

The effect of DFMO on the protein expression of GRP78, CHOP and cleaved caspase-12

To identify the direct effect of DFMO on ERS, the protein expression of GRP78, CHOP and cleaved caspase-12 were examined. Compared with the control group, ISO treatment

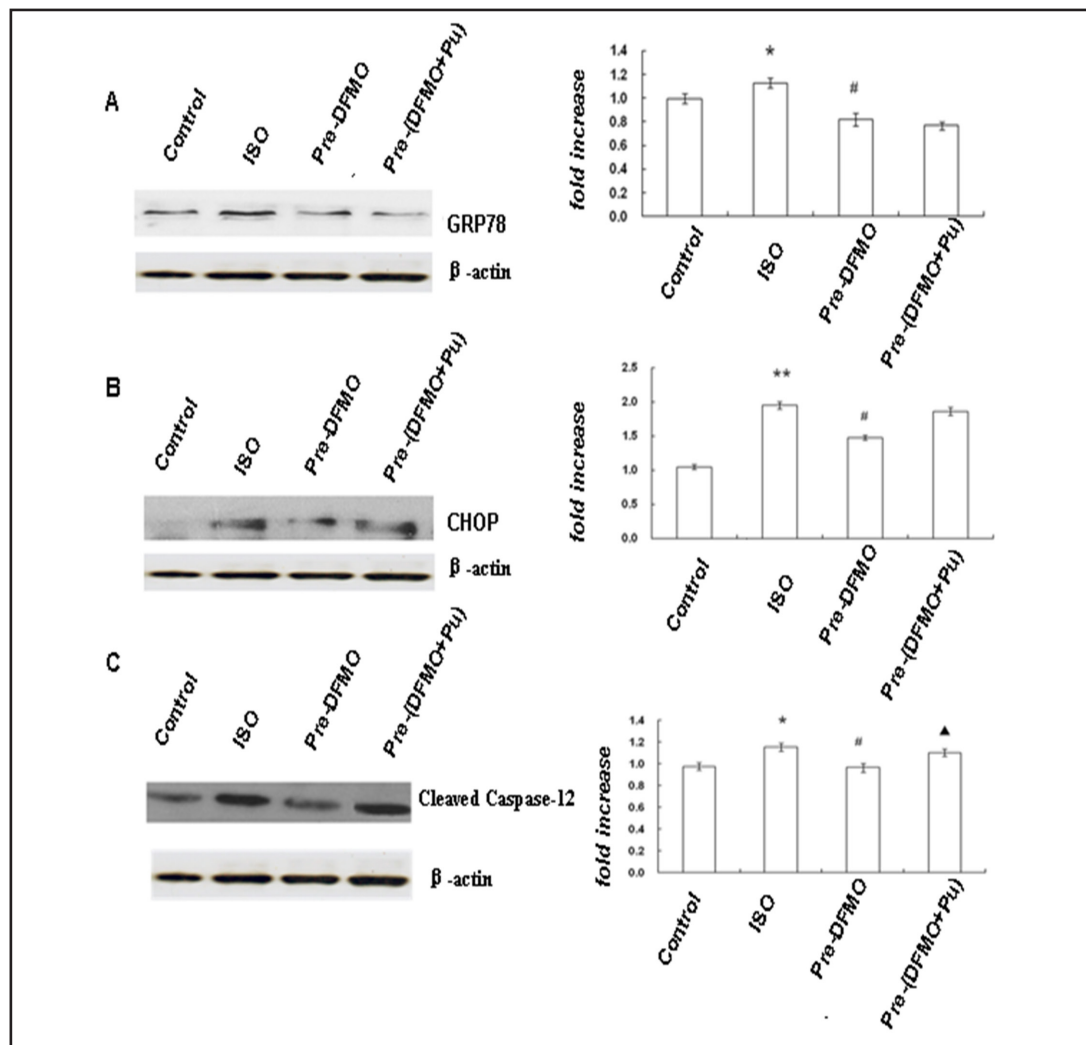


Fig. 6. DFMO inhibits ERS induced by ISO. Protein levels of ERS biomarkers GRP78 (A), CHOP (B), and cleaved caspase-12 (C) were determined by Western blot. The control group's protein levels were considered basal levels, and data from the other experimental groups are expressed as fold change from the control group. The fold change values represent the mean \pm S.E.M. of four determinations. * $P < 0.05$ or ** $P < 0.01$ versus the control group. # $P < 0.05$ versus ISO group. $\blacktriangle P < 0.05$ versus 0.5 mM pre-DFMO group.

increased the expression levels of GRP78 ($P < 0.05$), CHOP ($P < 0.01$) and cleaved caspase-12 ($P < 0.05$). DFMO reversed these increases ($P < 0.05$ versus ISO group). In contrast, the expression of GRP78 and CHOP were unchanged and the expression of cleaved caspase-12 was up regulated in the DFMO and Pu treatment group compared with the DFMO group ($P < 0.05$) (Fig. 6A-C).

Discussion

In response to pathophysiological stresses, cardiomyocytes undergo hypertrophic growth and/or apoptosis. Cardiac hypertrophy and apoptosis may play an important role in several cardiovascular diseases, such as ischemia/reperfusion, infarction, heart failure and aging. Naturally occurring polyamines are important cellular constituents essential for

growth, differentiation, and apoptosis, with cellular levels tightly regulated. ODC, the first rate-limiting enzyme in polyamine biosynthesis, converts ornithine to putrescine, putrescine is then converted to spermidine and spermine. SSAT then acetylates both spermine and spermidine to generate acetylated polyamines, which are subsequently converted to putrescine by polyamine oxidase [16, 17].

Increasing evidence has showed that the level of ERS is significantly elevated in hypertrophic cardiomyocytes [18]. If the anti-hypertrophic role of polyamine depletion is mediated by ERS, is yet to be determined. In the present study, we demonstrated that DFMO can attenuate ISO-induced hypertrophy and apoptosis in cardiomyocytes. Moreover, these protective effects appear to be mediated by ERS, which may involve Bcl-2/Bax regulation.

Cardiac hypertrophy is characterized by an increase in cell surface area, protein synthesis and activation of the fetal gene program (e.g. ANP). Several recent studies have demonstrated that growth responses in cardiac tissue are accompanied by increased polyamine levels. In addition, DFMO, an irreversible inhibitor of ODC, can reduce putrescine levels by inhibiting the activity of ODC; it has also been shown to inhibit hypertrophy and fibrosis-related genic responses in cardiac cells [19-21]. In this study, we observed that ISO increased cardiac hypertrophy and the rate of apoptosis, and DFMO pretreatment attenuated hypertrophy and apoptosis in cardiomyocytes. Furthermore, gene and protein levels of ODC and SSAT were increased in the ISO treated group. It is possible ISO may enhance polyamine synthesis through induction of ODC expression. It has been reported that β -adrenoceptor mediated hypertrophy *in vivo* occurs as a result of ODC induction [17]. It is also possible, however, that increased polyamines might influence cardiac hypertrophy and apoptosis through regulation of the ERS pathway. DFMO treatment did markedly decrease the gene and protein expression of ODC while increasing the gene and protein expression of SSAT. This effect was reversed by putrescine pretreatment. Previously our results have demonstrated that DFMO blocks polyamine synthesis through inhibiting the activity and expression of ODC, moreover, DFMO can decrease putrescine, spermidine and total polyamine pool. Our results have demonstrated that DFMO may play an anti-hypertrophic and anti-apoptotic role by inhibiting polyamine biosynthesis. These results are consistent with recent papers reporting a similar protective effect of DFMO against apoptosis in cardiac cells treated with aldosterone, or when cells were exposed to simulated ischemia conditions [20, 21]. A study investigating the effects of polyamine depletion on norepinephrine mediated apoptosis in rat neonatal cardiomyocytes and H₉C₂ cardiomyoblasts demonstrated that DFMO had a pro-survival effect that was mediated through a specific network of phosphatases and kinases [22].

GRP78 is considered an ERS activation biomarker, and CHOP is the canonical protein in ERS-induced apoptosis. CHOP-deficient cells are resistant to ERS-mediated apoptosis and the overexpression of CHOP can lead to cell cycle arrest and/or apoptosis [23]. Enhanced expression of cleaved caspase-12 is an indicator of ERS associated apoptosis has been observed in heart tissues of diabetic patients [24]. The CHOP and caspase-12 proteins are distal effectors of ERS response mediating apoptotic signals. There is extensive evidence indicating ERS markers, GRP78 and CHOP are increased in cases of cardiac hypertrophy and incidences of heart failure [25, 26]. ERS has been shown to lead to apoptosis in these processes and as a link between apoptosis, cardiomyocyte loss, ventricular remodeling, and deterioration of systolic performance in multiple experimental models [27, 28]. In this study we have demonstrated three critical points. One, we have shown that ISO induced cardiac hypertrophy in myocytes, this was accompanied by up regulation of GRP78, CHOP, and cleaved caspase-12. Two, DFMO down regulated the expression of GRP78, CHOP, and cleaved caspase-12. Three, the presence of exogenous putrescine reversed this DFMO-induced down regulation of ERS pathway markers. These findings suggest that DFMO inhibits the ERS pathway, thus attenuating cardiomyocyte hypertrophy and apoptosis.

Many studies have demonstrated that overexpression of CHOP leads to decrease Bcl-2 protein levels and inhibits the translocation of Bax protein from the cytosol to the mitochondria [29]. Overexpression of Bcl-2 or Bax knockout has been shown to block CHOP-

induced apoptosis [30]. Our experimental results have shown DFMO treatment to increase the expression of an anti-apoptotic gene (Bcl-2) and decrease the expression of a pro-apoptotic gene (Bax). These studies combined with our results suggests DFMO treatment inhibits ISO-induced cardiac hypertrophy and decreased expression of ERS markers, resulting in Bcl-2 and Bax expression changes, and subsequent decrease in apoptosis.

To our knowledge, this is the first study to assess the interaction between ODC mediated polyamine synthesis and ERS signaling in neonatal rat cardiomyocytes. These findings contribute to the elucidation of DFMO as a negative regulator in cardiac hypertrophy and the development of methodologies to explore therapeutics for cardiac diseases.

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Disclosure Statement

The authors declare no conflict of interest.

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