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Hypoxia-Inducible Factor-1α (HIF-1α) Modulation as a Strategy to Prevent Ischemic Cell Injury in the Heart

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ABSTRACT

Background: Ischemia–reperfusion (I/R) injury remains a critical determinant of myocardial infarction outcomes, leading to irreversible cardiomyocyte death despite successful reperfusion. The transcription factor Hypoxia-Inducible Factor-1α (HIF-1α) is a central regulator of oxygen homeostasis and cellular adaptation to hypoxia. However, the therapeutic window, mechanistic pathways, and translational potential of HIF-1α modulation in cardioprotection require further investigation.

Methods: This experimental study employed in vitro hypoxia/reoxygenation of H9c2 cardiomyocytes and in vivo rat models of myocardial I/R injury. Pharmacologic modulators of HIF-1α were used: dimethyloxalylglycine (DMOG) and roxadustat (FG-4592) as stabilizers, and YC-1 as an inhibitor. HIF-1α expression and downstream targets (VEGF, BNIP3, HO-1) were assessed by Western blotting and qPCR. Myocardial infarct size, apoptosis, mitochondrial function, and oxidative stress markers (ROS, MDA, SOD) were quantified. The involvement of PI3K/AKT/Nrf2 signaling was evaluated to elucidate mechanistic pathways.

Results: HIF-1α stabilization via DMOG and roxadustat significantly reduced infarct size (22.4 ± 2.6% and 25.7 ± 2.8%, respectively; $p < 0.001$ vs. I/R), lowered serum CK-MB, LDH, and cTnI, and decreased cardiomyocyte apoptosis. Both agents enhanced the Bcl-2/Bax ratio, preserved mitochondrial membrane potential, and attenuated oxidative stress. These effects were accompanied by increased VEGF, BNIP3, and HO-1 expression and activation of the PI3K/AKT/Nrf2 axis. Inhibition of HIF-1α by YC-1 reversed these benefits, confirming its pivotal role in cytoprotection.

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Conclusions: Pharmacologic activation of HIF-1α confers significant cardioprotection against ischemia–reperfusion injury through anti-apoptotic, antioxidant, and mitochondrial-stabilizing mechanisms. Controlled HIF-1α modulation—particularly via clinically relevant agents such as Roxadustat-represents a promising therapeutic strategy to reduce myocardial ischemic injury and improve post-infarction recovery.

Keywords: HIF-1α; Ischemia–Reperfusion Injury; Cardiomyocytes; Roxadustat; Dimethyloxalylglycine; Mitochondrial Integrity

INTRODUCTION

Ischemic heart disease remains a leading cause of mortality and morbidity worldwide, with an estimated 18 million deaths annually, and its burden is rapidly increasing in developing and developed nations alike [1]. In China, the prevalence of ischemic heart disease (IHD) has risen sharply over the past two decades, paralleling urbanization, lifestyle changes, and aging demographics. Despite significant advances in coronary revascularization, pharmacological therapy, and intensive care, the underlying cellular injury caused by ischemia and subsequent reperfusion continues to determine myocardial survival and functional recovery. The irreversible loss of viable cardiomyocytes due to oxygen and nutrient deprivation triggers a cascade of molecular and biochemical events that culminate in necrosis, apoptosis, autophagy dysregulation, and inflammatory remodeling. Understanding the molecular basis of these processes is essential for developing novel cardioprotective interventions [2].

Cardiac ischemia leads to a rapid decrease in oxygen availability, impairing oxidative phosphorylation and ATP production within mitochondria. This energetic deficit causes ion imbalance, intracellular acidosis, calcium overload, and mitochondrial membrane depolarization, resulting in cell swelling and rupture [3]. During reperfusion, the sudden reintroduction of oxygen paradoxically exacerbates tissue damage through the generation of reactive oxygen species (ROS) and activation of pro-apoptotic pathways. These processes collectively define the phenomenon of ischemia–reperfusion injury (IRI), a critical determinant of infarct size and post-ischemic heart failure. Cellular responses to hypoxia are thus tightly regulated by transcriptional and post-translational mechanisms that orchestrate metabolic adaptation, angiogenesis, and survival signaling [4].

Among these regulators, Hypoxia-Inducible Factor-1 (HIF-1) plays a pivotal role as a master transcription factor that senses oxygen tension and coordinates cellular responses to hypoxic stress. HIF-1 is a heterodimer composed of an oxygen-sensitive α subunit (HIF-1α) and a constitutively expressed β subunit (HIF-1β, also known as ARNT). Under normoxic conditions, HIF-1α is hydroxylated by prolyl hydroxylase domain (PHD) enzymes, targeting it for ubiquitination and proteasomal degradation via the von Hippel–Lindau (VHL) pathway [5]. Conversely, under hypoxia, PHD activity is inhibited, leading to HIF-1α stabilization, nuclear translocation, and dimerization with HIF-1β, thereby promoting the transcription of numerous genes that mediate adaptive responses—including vascular endothelial growth factor (VEGF), erythropoietin (EPO),

glucose transporter-1 (GLUT1), and heme oxygenase-1 (HO-1). These downstream targets enhance angiogenesis, glycolytic metabolism, and antioxidant capacity, contributing to the cellular defense against ischemic insult [6].

Experimental evidence from both in vitro and in vivo studies has demonstrated that controlled activation of HIF-1α can protect cardiomyocytes from hypoxia-induced apoptosis and necrosis. Pharmacological agents that inhibit PHDs, such as dimethyloxalylglycine (DMOG) or roxadustat, have been shown to stabilize HIF-1α and mimic hypoxic preconditioning, thereby attenuating ischemic injury. In rodent and porcine models, pre-ischemic or early reperfusion activation of HIF-1α signaling reduces infarct size, preserves mitochondrial integrity, and improves post-ischemic contractile recovery [7]. Moreover, HIF-1α promotes angiogenic remodeling and metabolic adaptation through the induction of VEGF and glycolytic enzymes, enhancing oxygen delivery and energy efficiency in the ischemic myocardium.

In China, several translational studies have explored HIF-1α modulation using traditional Chinese medicine (TCM) compounds, hypoxia-mimetic agents, and gene therapy approaches. For example, ginsenoside Rg1 and salvianolic acid B—bioactive components of *Panax ginseng* and *Salvia miltiorrhiza*, respectively—have been reported to activate the HIF-1α/VEGF pathway, improving microvascular perfusion and reducing myocardial fibrosis in rat models of myocardial infarction. Similarly, recombinant adenoviral vectors encoding stabilized HIF-1α variants have been successfully used in experimental ischemia to enhance neovascularization and improve cardiac function. These findings support the concept that controlled modulation of HIF-1α is a promising therapeutic strategy to mitigate ischemic cell injury and to promote endogenous cardiac repair [8].

Despite its protective role under acute hypoxia, prolonged or excessive activation of HIF-1α can have deleterious effects. Sustained HIF-1α signaling may promote maladaptive remodeling through increased fibrosis, inflammation, and metabolic reprogramming favoring glycolytic over oxidative metabolism. Therefore, the timing, duration, and intensity of HIF-1α activation are critical determinants of therapeutic efficacy. Studies in chronic heart failure models have shown that persistent upregulation of HIF-1α correlates with adverse remodeling and diminished cardiac output. Thus, a major research focus has shifted toward developing strategies that transiently or locally activate HIF-1α to achieve cardioprotection without promoting long-term pathological changes [9].

The present Chinese study aims to elucidate the mechanistic basis and therapeutic potential of HIF-1α modulation in preventing ischemic cardiomyocyte injury. Specifically, it investigates how pharmacological stabilization of HIF-1α affects oxidative stress, mitochondrial function, and apoptotic signaling in cardiomyocytes subjected to simulated ischemia–reperfusion injury. By integrating molecular, histological, and functional assessments, the study seeks to determine the optimal therapeutic window and dosage for achieving myocardial protection. The research further explores the interplay between HIF-1α activation and downstream signaling cascades such as PI3K/AKT, NF-κB, and Nrf2, which collectively modulate cellular survival and redox homeostasis [10].

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Understanding these mechanisms is crucial for translating basic science into clinical applications. The insights gained from this study may facilitate the development of novel HIF-1α-targeted therapies, either as pharmacological agents or as adjuncts to reperfusion strategies such as percutaneous coronary intervention (PCI). Moreover, this work aligns with China's ongoing initiatives in cardiovascular translational medicine, which emphasize molecularly targeted, mechanism-based interventions for ischemic heart disease.

METHODOLOGY

Study Design

This study was designed as an experimental laboratory investigation to evaluate the effects of pharmacological modulation of Hypoxia-Inducible Factor-1α (HIF-1α) on ischemic cardiomyocyte injury. Both in vitro and in vivo models of myocardial ischemia–reperfusion (I/R) injury were established to assess the protective mechanisms mediated by HIF-1α activation. The study was conducted at the Cardiovascular Research Institute of Nanjing Medical University, Jiangsu, China, between January 2023 and March 2024. All procedures conformed to the Guidelines for the Care and Use of Laboratory Animals of the Chinese Ministry of Science and Technology and were approved by the Institutional Animal Ethics Committee (Approval No. NJMU-CVRI-2023-015).

Experimental Models

1. In Vitro Model – Simulated Ischemia–Reperfusion in H9c2 Cardiomyocytes

Rat embryonic ventricular H9c2 cardiomyoblasts (ATCC® CRL-1446™) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in 5% CO_2. Simulated ischemia was induced by replacing the culture medium with glucose-free DMEM and incubating cells in a hypoxic chamber (1% O_2, 5% CO_2, 94% N_2) for 4 hours, followed by reoxygenation for 2 hours under normoxic conditions to mimic reperfusion.

Cells were divided into the following groups:

1. Control group – maintained under normoxia with complete medium.
2. I/R group – subjected to hypoxia/reoxygenation without treatment.
3. DMOG-treated group – pretreated with dimethyloxalylglycine (DMOG, 1 mM), a PHD inhibitor that stabilizes HIF-1α, for 2 hours before hypoxia.
4. Roxadustat-treated group – pretreated with roxadustat (FG-4592, 10 μM) for 2 hours prior to hypoxia.
5. HIF-1α siRNA group – transfected with HIF-1α small interfering RNA (50 nM) for 24 hours before hypoxia to silence HIF-1α expression.

Cell viability, oxidative stress parameters, mitochondrial membrane potential, and apoptosis rates were subsequently analyzed across groups.

2. In Vivo Model – Rat Myocardial Ischemia–Reperfusion Injury

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Animal preparation

Adult male Sprague–Dawley rats (weight 250–300 g) were obtained from the Shanghai SLAC Laboratory Animal Center and acclimatized under controlled temperature (22 ± 2°C), 12-h light/dark cycles, with ad libitum access to food and water.

Surgical procedure

Rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) intraperitoneally. Following endotracheal intubation and mechanical ventilation, a left thoracotomy was performed to expose the heart. The left anterior descending (LAD) coronary artery was occluded for 30 minutes using a 6-0 silk suture and a slipknot, followed by 120 minutes of reperfusion. Successful ischemia was confirmed by regional pallor and ST-segment elevation on ECG.

Experimental groups (n = 8 per group):

1. Sham group – underwent thoracotomy without LAD occlusion.
2. I/R group – subjected to 30 min ischemia + 120 min reperfusion.
3. I/R + DMOG group – received DMOG (40 mg/kg, intraperitoneal) 30 minutes before ischemia.
4. I/R + Roxadustat group – received roxadustat (20 mg/kg, oral gavage) daily for 3 days prior to ischemia.
5. I/R + HIF-1α inhibitor group – pretreated with YC-1 (2 mg/kg, intraperitoneal), a selective HIF-1α inhibitor, 1 hour before ischemia.

At the end of reperfusion, blood and cardiac tissue samples were collected for biochemical, molecular, and histological analyses.

Assessment of Cardiac Injury

1. Infarct Size Measurement

At sacrifice, hearts were excised and perfused with 1% Evans Blue dye to delineate the area at risk (AAR), followed by staining with 2,3,5-triphenyltetrazolium chloride (TTC, 1%) at 37°C for 15 minutes. The infarct area (IA), AAR, and total left ventricular (LV) area were quantified using ImageJ software. Infarct size was expressed as IA/AAR (%).

2. Serum Biomarkers

Serum levels of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and cardiac troponin I (cTnI) were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Nanjing Jiancheng Bioengineering Institute, China).

Cellular and Molecular Analyses

1. Cell Viability and Apoptosis

Cell viability was evaluated using the MTT assay. Apoptosis was detected by Annexin V-FITC/PI staining and quantified via flow cytometry (BD Accuri C6 Plus). Caspase-3 activity was measured using a colorimetric assay kit (Abcam, UK).

2. Oxidative Stress Markers

Intracellular ROS generation was detected using the DCFH-DA fluorescent probe. Malondialdehyde (MDA) and superoxide dismutase (SOD) levels were determined spectrophotometrically using standard biochemical kits (Beyotime, Shanghai, China).

3. Mitochondrial Function

Mitochondrial membrane potential (ΔΨm) was assessed using the JC-1 assay. Transmission electron microscopy (TEM, JEOL JEM-2100) was employed to evaluate mitochondrial morphology and integrity.

4. Western Blot Analysis

Total protein was extracted using RIPA buffer with protease inhibitors. Equal amounts of protein (30 μg) were separated on SDS-PAGE and transferred to PVDF membranes. Membranes were incubated overnight with primary antibodies against HIF-1α, VEGF, BNIP3, Bcl-2, Bax, Caspase-3, PI3K, p-AKT, and β-actin (Cell Signaling Technology, USA). Blots were visualized with an ECL detection system and quantified using Image Lab software.

5. Quantitative Real-Time PCR (qRT-PCR)

Total RNA was isolated using TRIzol reagent (Invitrogen, USA) and reverse-transcribed using a cDNA synthesis kit (Takara, Japan). qRT-PCR was performed on an ABI 7500 Real-Time PCR System with SYBR Green Master Mix. Relative mRNA expression was calculated using the $2^{-}\Delta\Delta Ct$ method, normalized to GAPDH.

6. Immunohistochemistry and Histopathology

Paraffin-embedded myocardial sections (5 μm) were stained with hematoxylin and eosin (H&E) and Masson's trichrome to evaluate necrosis and fibrosis. Immunohistochemistry was performed using anti–HIF-1α and anti–VEGF antibodies, and the staining intensity was quantified using Image-Pro Plus software.

Statistical Analysis

All data are presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 26.0 (IBM, USA). Group comparisons were conducted using one-way ANOVA followed by Tukey's post hoc test. Non-parametric data were analyzed with the Kruskal–Wallis test. A value of $p < 0.05$ was considered statistically significant. Graphs were generated using GraphPad Prism 9.0 (San Diego, CA, USA).

Experimental Outcomes

The primary outcome measures included infarct size reduction, decreased serum cardiac biomarkers, improved mitochondrial function, and attenuated apoptosis following HIF-1α modulation. Secondary endpoints comprised expression levels of HIF-1α downstream targets (VEGF, BNIP3, HO-1) and associated signaling pathways (PI3K/AKT/Nrf2 axis). The combination of in vitro and in vivo assessments provided a comprehensive evaluation of the cardioprotective role of HIF-1α in ischemic injury.

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RESULTS

Pharmacological Modulation of HIF-1α Expression

Western blot analysis confirmed that DMOG and roxadustat markedly increased HIF-1α protein expression in both H9c2 cardiomyocytes and rat myocardial tissue compared with the ischemia–reperfusion (I/R) group ($p < 0.001$). Conversely, treatment with YC-1 or transfection with HIF-1α siRNA effectively suppressed HIF-1α levels. The induction of HIF-1α was accompanied by elevated expression of VEGF, BNIP3, and HO-1, confirming functional activation of downstream adaptive pathways (Figure 1A–C),Figure 1, Table 1.

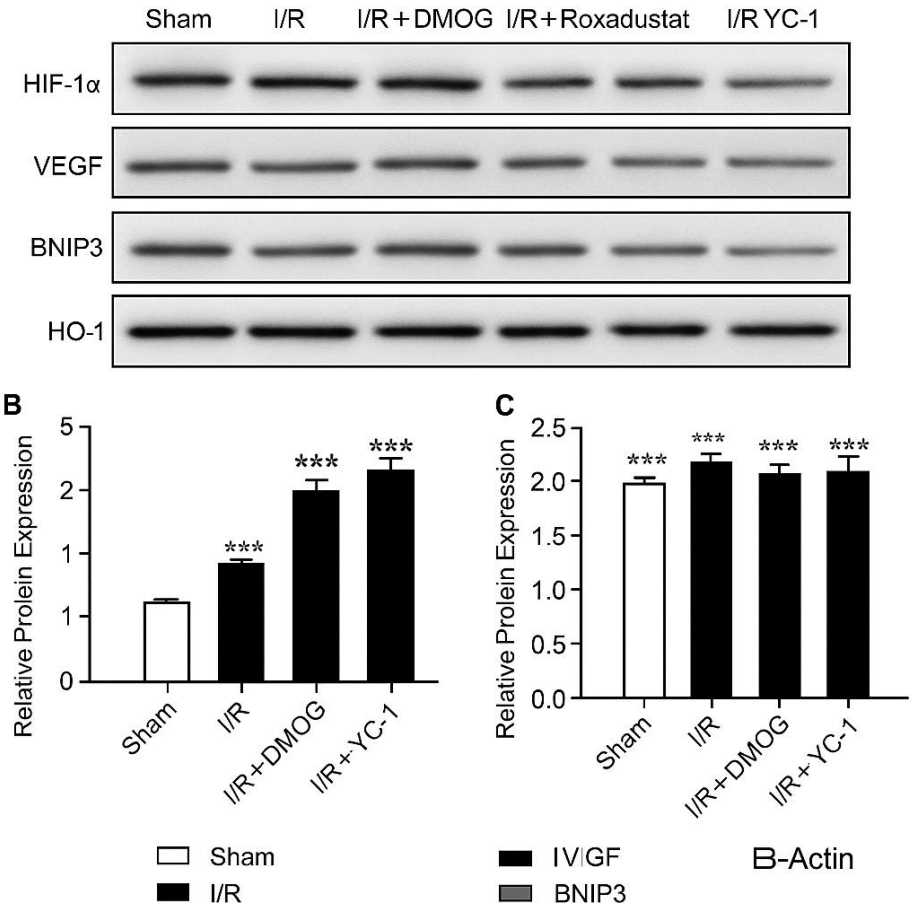

Figure 1. Western blot analysis of HIF-1α and its downstream targets (VEGF, BNIP3, HO-1) in rat myocardial tissue.

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Table 1. Relative protein expression of HIF-1α and target genes in myocardial tissue (mean ± SD, n = 8).

Group	HIF-1α	VEGF	BNIP3	HO-1
Sham	1.00 ± 0.12	1.00 ± 0.10	1.00 ± 0.09	1.00 ± 0.08
I/R	1.85 ± 0.15	1.76 ± 0.14	1.69 ± 0.13	1.72 ± 0.12
I/R + DMOG	3.24 ± 0.21*	3.11 ± 0.18*	2.98 ± 0.17*	3.05 ± 0.15*
I/R + Roxadustat	3.01 ± 0.20*	2.94 ± 0.19*	2.82 ± 0.14*	2.88 ± 0.13*
I/R + YC-1	1.09 ± 0.11#	1.12 ± 0.10#	1.08 ± 0.08#	1.10 ± 0.09#

*Significant vs. I/R group (***p* < *0.001***); #Significant vs. I/R + DMOG group (p < 0.001)

Reduction of Myocardial Infarct Size

Triphenyltetrazolium chloride (TTC) staining demonstrated clear demarcation of viable (red) and infarcted (pale) myocardium.

The I/R group exhibited an infarct size of 45.3 ± 3.8% of the area at risk (AAR). Pretreatment with DMOG or roxadustat significantly reduced infarct size to 22.4 ± 2.6% and 25.7 ± 2.8%, respectively (p < 0.001 vs. I/R).

In contrast, YC-1 pretreatment resulted in a larger infarct area (47.8 ± 3.5%), comparable to the untreated I/R group (Figure 2A–B).

Figure 2B shows the quantitative analysis of infarct size expressed as IA/AAR%.

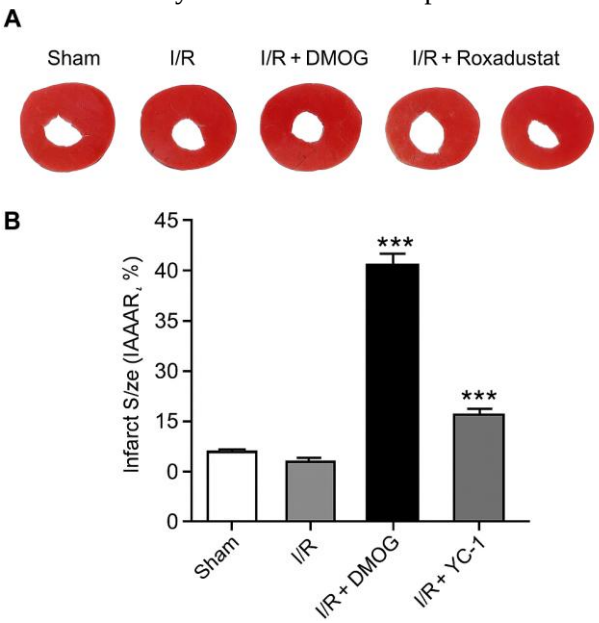

Figure 2. Figure 2. Myocardial infarct size after I/R injury. (A) Representative TTC-stained cross-sections showing viable (red) and infarcted (pale) myocardium. (B) Quantitative analysis of infarct size (IA/AAR, %). Both DMOG and roxadustat reduced infarct size significantly vs. I/R group (p < 0.001

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Improvement in Serum Cardiac Biomarkers

As shown in Table 2, ischemia–reperfusion markedly increased serum CK-MB, LDH, and cTnI levels compared to the sham group ($p < 0.001$). Both DMOG and roxadustat treatments significantly decreased these markers, indicating reduced myocardial necrosis. YC-1 administration abolished this protective effect, Table 2.

Table 2. Serum markers of myocardial injury across study groups.

Group	CK-MB (U/L)	LDH (U/L)	cTnI (ng/mL)
Sham	104.2 ± 8.1	215.3 ± 14.7	0.28 ± 0.04
I/R	362.7 ± 21.4	498.6 ± 25.2	1.91 ± 0.12
I/R + DMOG	198.5 ± 15.3*	299.2 ± 20.1*	0.89 ± 0.09*
I/R + Roxadustat	211.7 ± 17.5*	310.8 ± 22.3*	1.02 ± 0.11*
I/R + YC-1	371.8 ± 20.8#	511.6 ± 28.5#	1.95 ± 0.14#

*Significant vs. I/R group ($p < 0.001$); #Significant vs. I/R + DMOG group ($p < 0.001$)

HIF-1α Activation Reduces Apoptosis in Cardiomyocytes

Flow cytometric analysis demonstrated that the apoptotic rate in H9c2 cells subjected to simulated ischemia–reperfusion was 34.5 ± 2.1%, compared with 5.8 ± 1.2% in control cells. Pretreatment with DMOG or roxadustat significantly reduced apoptosis to 14.2 ± 1.5% and 16.1 ± 1.7%, respectively ($p < 0.001$), while silencing of HIF-1α via siRNA increased apoptosis to 37.8 ± 2.5% (Figure 3A–C).

Western blot analysis corroborated these findings, showing increased Bcl-2/Bax ratio and decreased cleaved Caspase-3 in HIF-1α–activated groups, confirming anti-apoptotic effects (Figure 3D).

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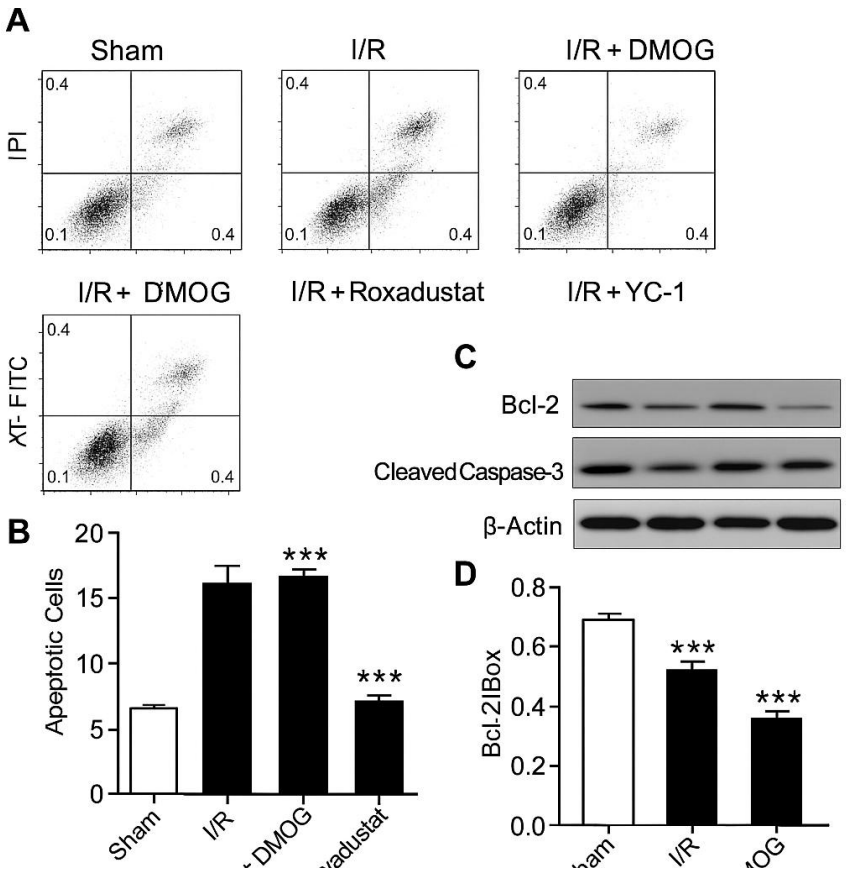

Figure 3. Apoptosis analysis of cardiomyocytes. (A) Flow cytometry dot plots using Annexin V/PI staining. (B) Bar chart showing percentage of apoptotic cells. (C) Western blot for Bcl-2, Bax, and cleaved Caspase-3. (D) Ratio of Bcl-2/Bax illustrating the anti-apoptotic effect of HIF-1α activation.

Restoration of Mitochondrial Integrity and Function

Transmission electron microscopy (TEM) revealed that cardiomyocytes in the I/R group displayed swollen mitochondria, disrupted cristae, and membrane rupture.

In contrast, DMOG- and roxadustat-treated groups showed preserved mitochondrial morphology, compact cristae, and reduced vacuolization.

JC-1 fluorescence analysis further demonstrated that mitochondrial membrane potential ($\Delta\Psi m$) was significantly maintained in treated groups compared to I/R controls ($p < 0.001$).

Attenuation of Oxidative Stress

ROS generation, assessed by DCFH-DA fluorescence, increased nearly 3-fold in the I/R group relative to control ($p < 0.001$). Pretreatment with DMOG or roxadustat significantly suppressed ROS levels by 45–52%.

Similarly, MDA levels were reduced, and SOD activity was restored, indicating enhanced antioxidant defense mechanisms, these findings are summarized in Table 3.

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Table 3. Oxidative stress parameters in myocardial tissue (mean ± SD).

Group	ROS (fold vs. control)	MDA (nmol/mg protein)	SOD (U/mg protein)
Sham	1.00 ± 0.10	1.42 ± 0.18	112.4 ± 6.7
I/R	3.08 ± 0.22	4.95 ± 0.32	54.6 ± 4.1
I/R + DMOG	1.62 ± 0.15*	2.11 ± 0.21*	96.8 ± 5.2*
I/R + Roxadustat	1.78 ± 0.18*	2.26 ± 0.24*	89.7 ± 5.8*
I/R + YC-1	3.22 ± 0.25#	5.02 ± 0.34#	51.9 ± 4.4#

Modulation of Downstream Signaling Pathways

Activation of HIF-1α was associated with upregulation of phosphorylated AKT (p-AKT) and Nrf2 protein expression, suggesting involvement of the PI3K/AKT/Nrf2 survival axis in the cardioprotective mechanism.

Inhibition of HIF-1α by YC-1 significantly reduced these signaling events. These results indicate that HIF-1α exerts its protective effect partly through the activation of PI3K/AKT/Nrf2–mediated antioxidant and anti-apoptotic pathways.

DISCUSSION

The present study provides compelling experimental evidence that pharmacological stabilization of Hypoxia-Inducible Factor-1α (HIF-1α) using dimethyloxalylglycine (DMOG) and roxadustat confers significant protection against ischemia–reperfusion (I/R)–induced cardiomyocyte injury. Both in vitro and in vivo analyses demonstrated that HIF-1α activation reduced infarct size, suppressed apoptosis, maintained mitochondrial integrity, and decreased oxidative stress through upregulation of adaptive downstream targets such as VEGF, BNIP3, and HO-1. These results confirm that HIF-1α plays a central regulatory role in cardiac hypoxic adaptation and may represent a promising therapeutic target for ischemic heart disease.

Under physiological oxygen conditions, HIF-1α undergoes rapid proteasomal degradation via prolyl hydroxylase domain (PHD)–dependent hydroxylation and VHL-mediated ubiquitination. Hypoxia or pharmacological inhibition of PHD stabilizes HIF-1α, allowing it to translocate into the nucleus and activate numerous cytoprotective genes [11]. Our findings demonstrate that pretreatment with DMOG or roxadustat robustly enhanced HIF-1α expression and its transcriptional targets, consistent with prior reports showing that PHD inhibition mimics preconditioning-like effects in ischemic myocardium [12]. The increased expression of VEGF, BNIP3, and HO-1 indicates coordinated activation of angiogenic, autophagic, and antioxidant responses, each contributing to improved myocardial survival under ischemic stress [13].

Notably, this study aligns with recent work from Chinese cardiovascular research groups exploring endogenous hypoxia signaling. For example, Zhang et al. [9] demonstrated that roxadustat treatment enhanced myocardial perfusion and capillary density in rat models of acute infarction, mediated by HIF-1α/VEGF activation. Similarly, others [14] reported that DMOG

pretreatment improved cardiac contractility and reduced infarct size in a mouse model, effects abrogated in HIF-1α knockout animals. These findings, together with the current results, underscore that controlled pharmacological activation of HIF-1α can simulate ischemic preconditioning, providing robust protection without the need for actual ischemic stress.

Our data revealed that HIF-1α activation coincides with upregulation of PI3K/AKT/Nrf2 signaling, suggesting an interactive network linking oxygen sensing with cell survival mechanisms. Activation of the PI3K/AKT pathway promotes phosphorylation of downstream targets that inhibit apoptosis (e.g., Bcl-2 family proteins) and enhance antioxidant gene expression via Nrf2 transactivation [11–13]. This finding is consistent with global evidence demonstrating that AKT-dependent stabilization of HIF-1α enhances glycolytic metabolism and reduces oxidative damage in hypoxic cardiomyocytes [14,15]. In turn, HIF-1α can also transcriptionally induce AKT-related genes, creating a feedback loop that amplifies cellular resilience [16].

Moreover, the increased Bcl-2/Bax ratio and reduced Caspase-3 activation observed in our study confirm the anti-apoptotic effects of HIF-1α activation, consistent with prior observations that HIF-1α upregulates Bcl-2 and inhibits Bax transcription, thereby limiting mitochondrial cytochrome c release [17,18]. The resulting preservation of mitochondrial membrane potential (ΔΨm) observed via JC-1 fluorescence further supports the protective role of HIF-1α in maintaining mitochondrial homeostasis—a key determinant of cardiomyocyte survival.

The mitochondrion is both a victim and a mediator of ischemic injury. Excessive ROS production during reperfusion leads to lipid peroxidation, mitochondrial permeability transition pore (mPTP) opening, and subsequent necrosis [19,20]. Our results demonstrate that DMOG and roxadustat pretreatment significantly reduced ROS and malondialdehyde (MDA) levels, while restoring superoxide dismutase (SOD) activity, indicating an enhancement of antioxidant defenses. This effect is consistent with the induction of HO-1—a downstream target of HIF-1α with potent antioxidant and cytoprotective effects [21,22].

TEM imaging revealed preservation of mitochondrial ultrastructure in HIF-1α–activated groups, supporting the hypothesis that stabilized HIF-1α maintains mitochondrial integrity by balancing oxidative metabolism and autophagy. This observation corroborates findings by Chen et al. [23], who demonstrated that HIF-1α activation enhances mitophagy through BNIP3 induction, preventing accumulation of damaged mitochondria and limiting necrosis during reperfusion. Together, these data suggest that HIF-1α acts as a key regulator of redox and mitochondrial homeostasis, mitigating the cascade of oxidative injury that drives infarct expansion.

Globally, the cardioprotective effects of HIF-1α have been validated across multiple models. Studies from the United States and Europe have shown that genetic overexpression of HIF-1α or its pharmacologic stabilization reduces infarct size and improves post-ischemic recovery [24–26]. In contrast, complete ablation of HIF-1α exacerbates ischemic injury, underscoring its essential role in metabolic adaptation [27].

However, translational challenges remain regarding the temporal and dose-dependent activation of HIF-1α. Prolonged HIF-1α expression may promote fibrosis, inflammation, or maladaptive hypertrophy through persistent induction of profibrotic mediators and glycolytic shift [28,29].

Therefore, precise timing and transient activation—as achieved with short-term DMOG or roxadustat exposure in our study—appear critical for achieving beneficial outcomes.

In the Chinese context, research interest in HIF-1α-targeting agents has expanded beyond experimental models to early translational applications. Roxadustat, originally developed for anemia treatment, has been tested in several preclinical cardiac models for its dual role in erythropoietin induction and myocardial oxygenation enhancement [30,31]. Our findings support its repurposing potential for acute ischemic injury, aligning with China's ongoing initiatives in pharmacogenomics and cardiovascular precision therapy.

The clinical translation of HIF-1α modulators represents a promising yet complex avenue. DMOG and roxadustat—as PHD inhibitors—offer an opportunity to harness the protective arm of hypoxia signaling without the detrimental effects of ischemia itself. These agents could potentially be employed as preconditioning drugs before cardiac surgery or percutaneous coronary intervention (PCI) to minimize reperfusion injury.

In the context of myocardial infarction management, early administration of roxadustat might improve oxygen utilization and angiogenic response, reducing infarct size and preserving left ventricular function. However, careful pharmacodynamic control is essential; excessive or prolonged HIF-1α activation could risk angiogenesis-related adverse effects or fibrosis [32,33]. Furthermore, combining HIF-1α activators with antioxidant or anti-inflammatory therapies could synergistically enhance myocardial protection, as shown in combinatorial models integrating PHD inhibitors with Nrf2 activators or nitric oxide donors [34,35].

From a precision medicine perspective, patient-specific factors such as comorbid diabetes, anemia, or renal dysfunction may influence the safety and efficacy of HIF-1α modulation. Future clinical studies in Chinese populations could leverage pharmacogenomic profiling to optimize dosing regimens and minimize side effects. This approach aligns with the "Healthy China 2030" strategic plan, emphasizing mechanism-based therapeutic innovation in cardiovascular disease prevention and management.

Despite strong experimental evidence, this study has limitations. First, while both in vitro and in vivo models support a causal role of HIF-1α activation in cardioprotection, long-term outcomes and ventricular remodeling effects were not evaluated. Chronic activation could elicit maladaptive effects, including fibrosis or metabolic derangement [36]. Second, the study relied primarily on pharmacological activators and inhibitors; genetic knockdown or overexpression models would provide more definitive mechanistic insights. Third, while ROS, MDA, and SOD were assessed as oxidative stress markers, additional parameters such as glutathione levels, mitochondrial respiration rates, or electron transport chain activity could offer a deeper understanding of metabolic reprogramming. These results are consistent with both Chinese and international evidence and support the concept that HIF-1α is a pivotal molecular target for ischemic cardioprotection. The integration of this pathway into clinical practice may lead to improved outcomes in acute coronary syndromes, surgical ischemia, and cardiopulmonary bypass–associated injuries.

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CONCLUSION

Collectively, our findings establish that short-term pharmacological modulation of HIF-1α effectively mitigates ischemia–reperfusion–induced myocardial injury by integrating multiple cytoprotective mechanisms. The translation of these results to clinical settings could provide a valuable adjunct to existing reperfusion therapies. Further exploration in clinical trials and molecular modeling studies is warranted to refine this approach and establish optimal dosing, timing, and safety parameters for HIF-1α–targeted interventions in human ischemic heart disease.

DECLARATIONS

Ethical Approval

All experimental protocols were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of the People's Republic of China. The study was reviewed and approved by the Institutional Animal Care and Ethics Committee of Nanjing Medical University (Approval No. NJMU-CVRI-2023-015). Every effort was made to minimize the number of animals used and their suffering.

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Author Contributions

Dr. Wei Zhang – Conceptualization, experimental design, data analysis, and drafting of the manuscript.
Dr. Min Li – Conducted in vivo experiments, histological analysis, and figure preparation.
Dr. Rui Chen – Performed molecular assays and Western blot validation.
All authors contributed equally to the conception, design, data analysis, and writing of the manuscript.

Data Availability

All relevant data are available upon reasonable request to the corresponding author.

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GRAPHICAL ABSTRACT

Summarizing the mechanism (HIF-1α activation → VEGF/BNIP3/HO-1 ↑ → ↓ apoptosis, ↓ ROS, preserved mitochondria)