

Evaluating Semaglutide's Protection in H/R - Injured AC16 Cardiomyocytes: Oxidative Stress, Inflammation, Apoptosis, and Autophagy Insights

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Objective: Semaglutide shows potential in cardiovascular protection, yet its specific role and mechanism in H/R injury are unclear. Given the role of autophagy in cardiomyocyte protection and the unclear mechanism of semaglutide in H/R - induced injury, this study aims to assess semaglutide's protective effects on AC16 cardiomyocytes in an H/R model and probe its mechanism, focusing on autophagy.

Methods: AC16 cardiomyocytes were subjected to H/R to simulate H/R injury. Cells were divided into five groups: Control, H/R, H/R+Semaglutide, H/R+ Semaglutide+Rapamycin (autophagy activator), and H/R+Semaglutide+3-Methyladenine (3-MA, autophagy inhibitor). Cell viability, injury (LDH release), oxidative stress (MDA, SOD), and inflammation (IL-6, TGF- β) were assessed. Protein levels of cleaved caspase-3, caspase-9, Bax, Bcl-2, and the autophagy-related protein FUNDC1 were analyzed by Western blot.

Results: H/R treatment significantly decreased cell viability and increased LDH release, indicating severe cellular injury ($P < 0.05$). Semaglutide treatment effectively restored cell viability and reduced LDH release. Furthermore, Semaglutide significantly attenuated H/R-induced oxidative stress, as shown by decreased MDA levels and restored SOD activity ($P < 0.05$). The inflammatory response, characterized by elevated IL-6 and TGF- β , was also markedly suppressed by Semaglutide ($P < 0.05$). Flow cytometry analysis revealed that Semaglutide significantly reduced the apoptosis rate. Western blotting confirmed that Semaglutide downregulated pro-apoptotic proteins (cleaved caspase-3, caspase-9, Bax) and upregulated the anti-apoptotic protein Bcl-2. Importantly, Semaglutide increased the expression of FUNDC1. The protective effects were enhanced by Rapamycin and attenuated by 3-MA, indicating that autophagy is involved in the cardioprotection.

Conclusion: Semaglutide shows substantial protective efficacy in safeguarding AC16 from H/R-triggered injury. It operates by mitigating oxidative stress, dampening inflammatory processes, inhibiting apoptotic pathways. Importantly, this investigation revealed that the cardioprotective effect, to a considerable degree, mediated through its promotion of autophagy.

Keywords: semaglutide, hypoxia/reoxygenation, oxidative stress, inflammatory response, apoptosis, autophagy, FUNDC1

Introduction

Hypoxia/reoxygenation (H/R) injury remains a leading cause of mortality worldwide. It is characterized by a cascade of pathological events, including oxidative stress, inflammation, and cardiomyocyte apoptosis, which collectively impair cardiac function and promote heart failure.^{1,2} Despite advances in therapeutic strategies, such as ischemic preconditioning and pharmacological interventions, effective cardioprotective agents targeting these interconnected mechanisms remain limited, necessitating exploration of novel therapeutic targets^{3,4} and highlighting the need for agents that modulate multiple protective mechanisms simultaneously. In recent years, glucagon - like peptide - 1 receptor agonists (GLP - 1RAs) have emerged as a promising class of drugs for their potent cardiovascular protective effects, extending



beyond glycemic control.^{5–7} Among them, semaglutide—a next-generation weekly GLP-1RA formulation—has demonstrated remarkable reductions in major adverse cardiovascular events (MACE) in large-scale clinical trials: the LEADER trial reported a 13% relative risk reduction in MACE (including cardiovascular death, non-fatal myocardial infarction, and stroke) in patients with type 2 diabetes,⁸ while SUSTAIN-6 showed a 26% reduction in cardiovascular death and 21% reduction in non-fatal myocardial infarction over 2 years.⁹ Their protective mechanisms go beyond glycemic control, involving direct effects on myocardial cells through multiple targeted interventions. Myocardial ischemia-reperfusion injury and repair involve various pathophysiological processes; this vital process for saving ischemic myocardium often causes additional damage, such as cardiomyocyte apoptosis, inflammation, and oxidative stress, severely affecting cardiac function and patient prognosis.^{3,10–14} In the field of myocardial ischemia - reperfusion injury, several recent studies have shed light on its complex pathophysiology and potential therapeutic strategies. Zhu Q et al¹⁵ found that semaglutide inhibits ischemia/reperfusion-induced cardiomyocyte apoptosis by activating the PKG/PKC ϵ /ERK1/2 pathway. Another study by Liu Y et al¹⁶ demonstrated that semaglutide attenuates H/R by inhibiting ferroptosis of cardiomyocytes via activation of the PKC - S100A9 axis. Notably, our prior study revealed that semaglutide protects AC16 cardiomyocytes from H/R injury by activating PINK1/PARKIN-mediated autophagy, a critical cellular quality control mechanism.¹⁷ Despite progress in GLP-1RA research, the molecular mechanisms underlying semaglutide's cardioprotective effects remain incompletely understood, particularly in human cardiomyocytes. The precise interplay between autophagy and other key protective pathways (eg, antioxidant defense, anti-inflammation, anti-apoptosis) remains poorly characterized. Specifically, it is unclear whether autophagy activation enhances or modulates the efficacy of other protective mechanisms, or if these pathways operate independently. This study systematically dissects semaglutide's cardioprotective mechanisms in H/R-injured AC16 cells. We hypothesize that semaglutide attenuates H/R - induced cardiomyocyte injury through a coordinated mechanism involving enhanced cell viability, reduced oxidative damage, suppressed inflammatory responses, and inhibited apoptosis, with autophagy serving as a regulatory hub.

To test this, we employed pharmacological tools (RAPA as an autophagy enhancer and 3MA as an inhibitor) and quantified oxidative stress, inflammation and apoptosis. Our findings will reveal novel synergistic mechanisms of semaglutide-mediated cardioprotection, supporting its potential as a multifaceted therapy for H/R injury.

Materials

Cells

The AC16 cardiomyocyte cell line was purchased from the China National Center for Cell Line Preservation and Management (Chinese Academy of Sciences).

Drugs and Reagents

Semaglutide was obtained from MCE (HY-114118). Rapamycin (RAPA) and 3-methyladenine (3-MA) were purchased from Sigma-Aldrich (Catalog Nos. R5000 and M9281, respectively). Penicillin-streptomycin was sourced from Solarbio (Catalog No. P1400). Dulbecco's Modified Eagle Medium (DMEM) was obtained from SIGMA (Catalog No. D6429-500ML), and fetal bovine serum (FBS) was purchased from DQ (Catalog No. YSN0121). The CCK-8 assay kit was from Solarbio (Catalog No. CA1210). ELISA kits for transforming growth factor- β 1 (TGF- β 1) and interleukin-6 (IL-6) were obtained from Solarbio (Catalog No. SEKH-0002) and R&D Systems (Catalog No. DLB50), respectively. Colorimetric assay kits for lactate dehydrogenase (LDH), malondialdehyde (MDA), and superoxide dismutase (SOD) were purchased from Elabscience (Catalog Nos. E-BC-K046-M, E-BC-K028-M, and E-BC-K022-M, respectively). Other experimental materials included cell culture flasks and 96-well plates from KIRGEN (T25 flasks and 96-well plates), sterile PBS from Vivacell (Catalog No. C3580-0500), and dimethyl sulfoxide (DMSO) from Solarbio (Catalog No. D8371). The Caspase-3 antibody (dilution ratio 1:1000, catalog number ER30804) was procured from HuaAn Biotechnology; the Caspase-9 antibody (dilution ratio 1:1000, catalog number GTX112888) was acquired from Gene Tex; the β -actin antibody (dilution ratio 1:5000, catalog number EM21002) was obtained from HuaAn Biotechnology; the Bax antibody (dilution ratio 1:1000, catalog number ET1603-34) was also purchased from HuaAn Biotechnology; and the BCL-2 antibody (dilution

ratio 1:1000, catalog number ARG55188) was sourced from arigo, and the FUNDC1 antibody (dilution ratio 1:1000, catalog number AF0002) was sourced from Affinity.

Instruments

A constant-temperature incubator (Thermo Scientific Forma series) and a carbon dioxide cell culture incubator (CI-191C, Suzhou Jiemei) were used. Flow cytometry was performed using a FACSCanto™ BD instrument.

Experimental Methods

Subculture and Culture of AC16 Cardiomyocytes

AC16 cells were removed from a -80°C freezer and rapidly thawed in a 37°C water bath. Once the cells reached a slushy state, they were quickly transferred to pre-warmed DMEM containing 10% FBS and 1% penicillin-streptomycin (P/S), centrifuged at 1000 rpm for 3 minutes, and the supernatant was discarded. The cell pellet was resuspended in 1 mL of culture medium and transferred to a T25 culture flask for incubation at 37°C in a humidified atmosphere with 5% CO_2 . The cells were passaged every two days at a 1:3 ratio and used for experiments when they reached the exponential growth phase.

Construction of the AC16 Cardiomyocyte Hypoxia/Reoxygenation Model

To simulate myocardial H/R injury, AC16 cardiomyocytes were cultured under hypoxic conditions (94% N_2 , 5% CO_2 , and 1% O_2 at 37°C) overnight, followed by reoxygenation under normoxic conditions (95% air and 5% CO_2 at 37°C) for 12 hours to establish the H/R injury model.⁵ Control cells were cultured under standard DMEM conditions.

Model Validation

Cell Viability Assessment

After the H/R treatment, cell viability was measured using the CCK - 8 assay. Briefly, 10 μL of CCK - 8 reagent was added to each well of the cell culture plate containing the H/R - treated and control cells. The cells were then incubated for an additional 2 hours at 37°C . Absorbance was measured at 450 nm using a microplate reader. A significant decrease in cell viability in the H/R - treated group compared to the control group indicated successful induction of cell injury, which is a characteristic feature of ischemia - reperfusion injury.

LDH Release Measurement

LDH release into the culture medium is an indicator of cell membrane damage and cell death. LDH release was quantified according to the manufacturer's instructions for the LDH detection kit. Higher levels of LDH in the culture medium of the H/R - treated cells compared to the control cells further confirmed the successful establishment of the H/R injury model, as increased LDH release is typically associated with cell damage during ischemia - reperfusion.

Grouping and Drug Treatment

As demonstrated in our prior study,¹⁷ a dose - response experiment was conducted by CCK-8 using a range of semaglutide concentrations (ranging from 1.25 mmol/L to 10 mmol/L), the optimal concentration was determined to be 5 mmol/L. Hence, during the reoxygenation phase, experimental cells were treated with 5 mmol/L concentrations of semaglutide. Additionally, groups treated with rapamycin (RAPA, an autophagy activator, 0.1 μM) and 3-methyladenine (3MA, an autophagy inhibitor, 5 mM) were established to evaluate the role of autophagy in H/R-induced cardiomyocyte injury.

CCK-8 and LDH Assays

For the CCK-8 assay, 10 μL of CCK-8 reagent was added to each well after drug treatment, and the cells were incubated for an additional 2 hours. Absorbance was measured at 450 nm using a microplate reader. LDH release was quantified according to the manufacturer's instructions for the LDH detection kit.

Oxidative Stress Marker Assay

Oxidative stress refers to a state in which the balance between the production of reactive oxygen species (ROS) and the body's antioxidant defense system is disrupted, leading to an excessive accumulation of ROS. This can cause damage to cellular components such as lipids, proteins, and DNA. In this study, intracellular malondialdehyde (MDA) content and superoxide dismutase (SOD) activity were measured using MDA and SOD detection kits, respectively, to assess oxidative stress levels.

Flow Cytometric Analysis of Apoptosis

Apoptosis is a programmed cell death process that is tightly regulated by a series of genes. It is characterized by specific morphological and biochemical changes, including cell shrinkage, chromatin condensation, and the formation of apoptotic bodies. In this study, apoptosis was detected using an Annexin V-FITC/PI double-staining apoptosis kit according to the manufacturer's instructions.

Western Blot Analysis

Cell pellets were collected, lysed in RIPA buffer containing PMSF, and centrifuged to obtain the supernatant. Protein concentration was determined using the BCA method, and proteins were denatured by boiling after equalization with TBST. Proteins were separated by SDS-PAGE gel electrophoresis and transferred to PVDF membranes using a semi-dry transfer system. After blocking with BSA and washing, the membranes were incubated overnight with specific primary antibodies against Bcl-2 (1:1000, Arigo, ARG55188), Bax (1:1000, HuaAn Biotechnology, ET1603-34), caspase-3 (1:1000, HuaAn Biotechnology, ER30804), caspase-9 (1:1000, Gene Tex, GTX112888), FUNDC1 (1:1000, Affinity, AF0002) followed by incubation with secondary antibodies the next day. Membranes were washed three times with TBST and incubated for 1 h at room temperature with HRP-conjugated secondary antibodies (diluted 1:10,000 in blocking buffer), including goat anti-mouse IgG-HRP (for β -actin, if the primary antibody was mouse-derived) and goat anti-rabbit IgG-HRP (for Bcl-2, Bax, caspase-3, caspase-9, and FUNDC1 as all are rabbit-derived primary antibodies).

After adding the ECL developing solution, the membranes were visualized using an imaging system, and the optical density values of the target bands were analyzed using a gel image processing system.

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 software. Measurement data are presented as mean $\pm$ standard deviation ($\bar{X} \pm S$). Comparisons across multiple groups were made using one-way ANOVA, and Tukey's post hoc test was used for subsequent pairwise analyses. All experiments were performed with at least 3 independent biological replicates ($n = 3$). In addition, all P values were evaluated using a two-sided test, and $P < 0.05$ was considered statistically significant.

Results

CCK-8 Activity and LDH Activity

Compared to the control group, AC16 cardiomyocyte CCK8 viability was significantly reduced in the H/R treatment group ($P < 0.05$), while LDH release was significantly increased ($P < 0.0001$), indicating cell damage induced by H/R. In the H/R + Semaglutide treatment group, cell viability was significantly restored ($P < 0.05$), and LDH release was significantly decreased ($P < 0.01$), demonstrating the significant protective effect of semaglutide against H/R-induced cardiomyocyte injury. When the autophagy activator RAPA was added to the semaglutide treatment, cell viability further increased ($P < 0.05$), and LDH release further decreased ($P < 0.05$), suggesting that RAPA enhanced the protective effect of semaglutide. Conversely, when the autophagy inhibitor 3-MA was added, cell viability decreased ($P < 0.05$), and LDH release increased ($P < 0.05$), indicating that 3-MA partially reversed the protective effect of semaglutide (Table 1 and Figure 1).

Table 1 Comparison of Cell Viability of AC16 Cells in Different Groups

Group	Cell Viability (%)
Control	100.00±0.00
H/R	64.94±6.13 ^a
H/R+semaglutide	82.62±8.42 ^{a,b}
H/R+semaglutide+RAPA	90.62±10.08 ^{a,b,c}
H/R+semaglutide+3MA	71.35±6.58 ^{a,b,c}

Notes: Data were represented in mean ± SEM.

^arepresents significance from the control group ($P<0.05$);

^brepresents significance from the H/R group ($P<0.05$);

^crepresents significance from the H/R+semaglutide group ($P<0.05$).

Inflammatory Response Assessment

H/R treatment significantly increased the expression of inflammatory cytokines IL-6 and TGF- β 1 ($P<0.0001$), indicating the induction of an inflammatory response by H/R. Compared to the H/R group, the semaglutide treatment group showed significantly reduced expression of IL-6 and TGF- β 1 ($P<0.05$), demonstrating the ability of semaglutide to alleviate H/R-induced inflammation. In the semaglutide + RAPA combination treatment group, the expression of IL-6 and TGF- β 1 was further reduced ($P<0.001$ for both), suggesting that RAPA enhanced the anti-inflammatory effect of semaglutide. Conversely, in the semaglutide + 3-MA combination treatment group, the expression of IL-6 and TGF- β 1 increased ($P<0.05$ and $P<0.001$, respectively), indicating that 3-MA partially reversed the anti-inflammatory effect of semaglutide (Figure 2).

Oxidative Stress Assessment

H/R treatment led to a significant increase in MDA content ($P<0.0001$) and a significant decrease in SOD activity ($P<0.0001$), indicating the induction of oxidative stress by H/R. Semaglutide treatment significantly reduced MDA

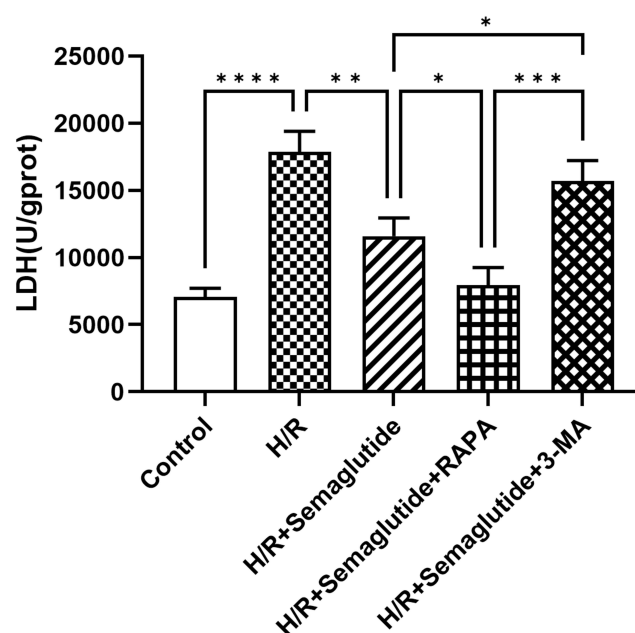


Figure 1 Comparison of LDH levels across experimental groups. Semaglutide decreased the level of LDH. Data are expressed as the mean ± standard deviation of three independent experiments.

Notes: * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

Abbreviations: RAPA, rapamycin; 3-MA, 3-methyladenine; H/R, hypoxia/reoxygenation; LDH, lactate dehydrogenase-I.

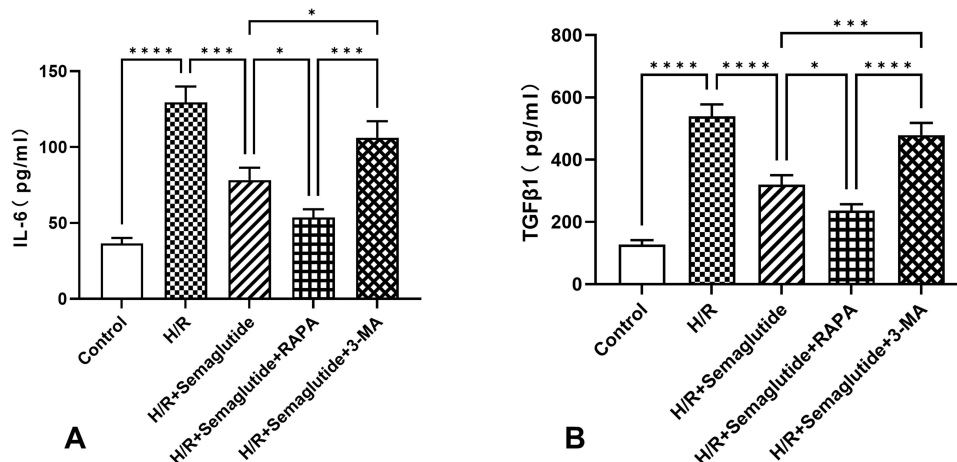


Figure 2 Effects of Semaglutide on IL-6 and TGF-β1 Levels in AC16 Cells after H/R Treatment. (A) IL - 6 levels in AC16 cells under different treatment scenarios; (B) TGF -β1 levels in AC16 cells under different treatment conditions.

Notes: * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$.

Abbreviations: RAPA, rapamycin; 3-MA, 3-methyladenine; H/R, hypoxia/reoxygenation.

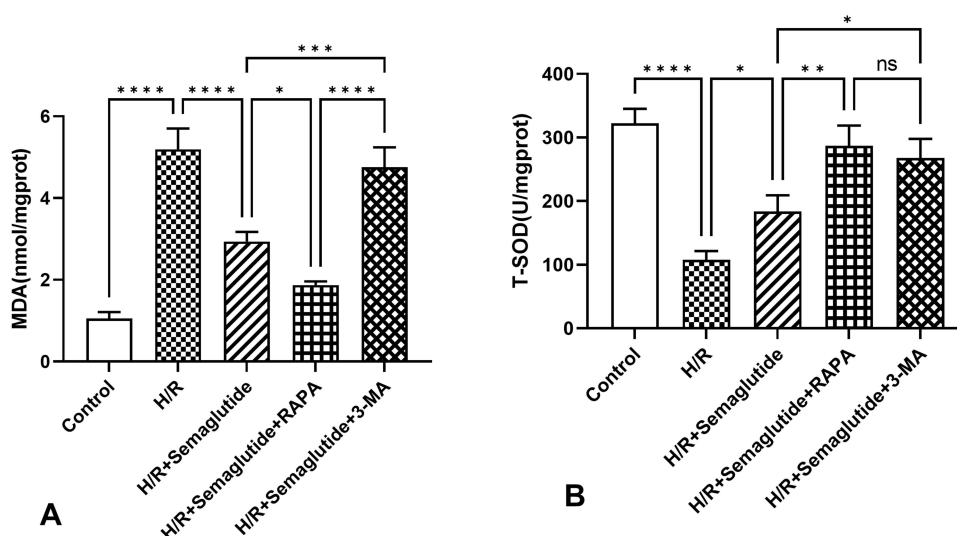


Figure 3 Effects of Semaglutide on MDA and SOD Levels in AC16 Cells after H/R Treatment. (A) MDA level: This figure presents the malondialdehyde (MDA) levels, a key indicator of lipid peroxidation caused by oxidative stress. (B) This graph shows the superoxide dismutase (SOD) levels in various experimental groups.

Notes: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Abbreviations: RAPA, rapamycin; 3-MA, 3-methyladenine; H/R, hypoxia/reoxygenation; ns, no significance.

content ($P < 0.0001$) and increased SOD activity ($P < 0.05$), demonstrating its ability to alleviate H/R-induced oxidative stress. Combination treatment with RAPA and semaglutide further reduced MDA content ($P < 0.05$) and increased SOD activity ($P < 0.01$), while combination treatment with 3-MA and semaglutide partially reversed these effects ($P < 0.05$) (Figure 3).

Myocardial Cell Apoptosis Rate

Under H/R conditions, AC16 cells exhibited a notable increase in apoptosis, as evidenced by flow cytometry analysis. Treatment with semaglutide significantly reduced the number of apoptotic cells, suggesting its anti-apoptotic effect. Rapamycin further decreased apoptosis levels, while 3-MA treatment reversed the protective effect of semaglutide, indicating its pro-apoptotic role (Figure 4). Flow cytometry analysis of the cell cycle revealed that H/R treatment induced cell cycle arrest, mainly in the G0/G1 phase. Semaglutide treatment reduced this cell cycle arrest, allowing cells to

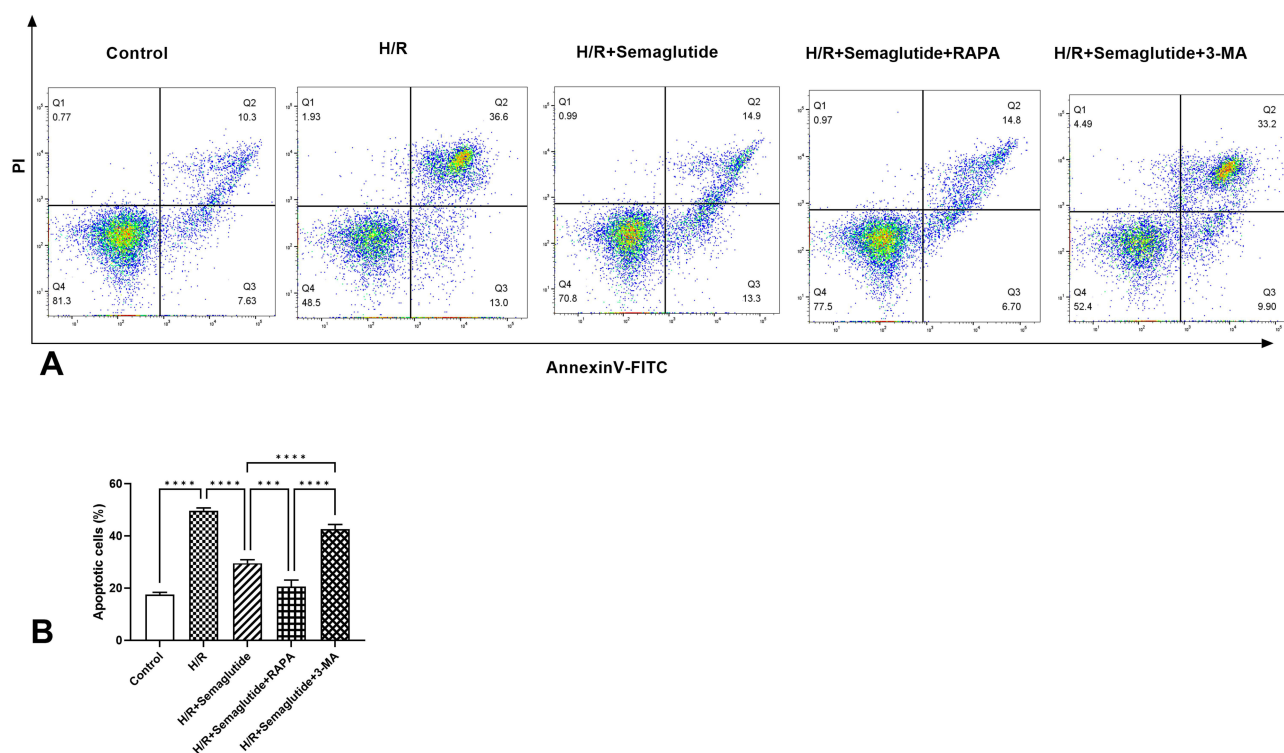


Figure 4 Semaglutide attenuated H/R-induced apoptosis tested by the Annexin V/PI assay. **(A)** Semaglutide decreased apoptotic rate tested by flow cytometry. **(B)** Statistical analysis of apoptosis was determined by the flow cytometric evaluation. The total apoptotic cell ratio was the sum of cells undergoing early apoptosis (lower right) and late apoptosis (upper right).

Notes: Q1-normal cells, Q2-early apoptosis, Q3-late apoptosis, Q4-dead cells. The calculation is based on Q2+Q3; *** $P < 0.001$, **** $P < 0.0001$.

Abbreviations: RAPA, rapamycin; 3-MA, 3-methyladenine; H/R, hypoxia/reoxygenation.

progress through the cell cycle more normally. This suggests that semaglutide can help mitigate the inhibitory effects of H/R on cell proliferation.

Western Blot Analysis of Apoptosis-Related Proteins

To examine the protective effect of semaglutide pretreatment on cardiac apoptosis, Western blot analysis was used to measure the expression of proteins related to apoptosis. The results showed that H/R treatment increased the levels of pro-apoptotic proteins caspase-3, caspase-9, and Bax, while reducing the level of the anti-apoptotic protein Bcl-2 ($P < 0.0001$). Compared to the H/R group, the H/R + Semaglutide pretreatment group displayed lower levels of caspase-3 and caspase-9, decreased Bax expression ($P < 0.0001$), and higher Bcl-2 levels ($P < 0.001$). These effects were even more notable in the H/R + Semaglutide + RAPA combination treatment group, while 3-MA partly reversed the inhibitory influence of semaglutide (Figure 5).

Expression of Autophagy-Related Proteins

Western blot analysis revealed that the expression level of the autophagy-related protein FUNDC1 was significantly elevated after hypoxia/reoxygenation (H/R) treatment ($P < 0.05$), indicating that the autophagy pathway was activated in response to H/R-induced injury. Treatment with Semaglutide further increased the expression of FUNDC1 ($P < 0.01$), suggesting that Semaglutide may exert a protective effect by enhancing the autophagy pathway.

The group co-treated with RAPA and Semaglutide exhibited the highest expression level of FUNDC1 ($P < 0.05$), indicating that RAPA augmented the activation of the autophagy pathway. Conversely, in the group co-treated with 3-MA and Semaglutide, the expression level of FUNDC1 was significantly reduced ($P < 0.05$), indicating that 3-MA inhibited autophagy activation (Figure 6).

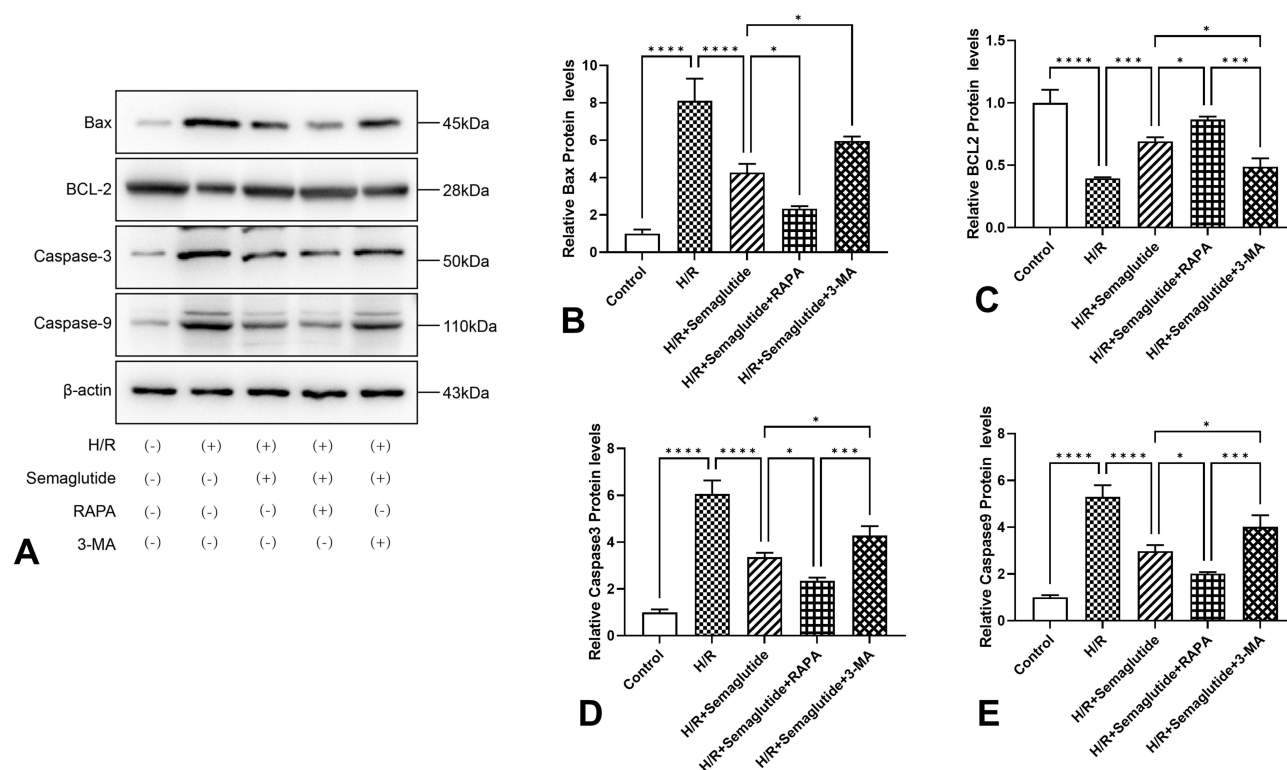


Figure 5 Semaglutide decreased cleaved caspase 3, caspase-9, Bax, increased the Bcl-2. **(A)** The bands of Bcl-2, Bax and Caspase-3, caspase-9 and their expression were detected using Western blotting. Quantitative analysis of Bcl-2 **(B)**, Bcl-2 **(C)**, caspase-3 **(D)**, caspase-9 **(E)**. The results were expressed as mean $\pm$ SD (n = 3).

Notes: * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$.

Abbreviations: RAPA, rapamycin; 3-MA, 3-methyladenine; H/R, hypoxia/reoxygenation.

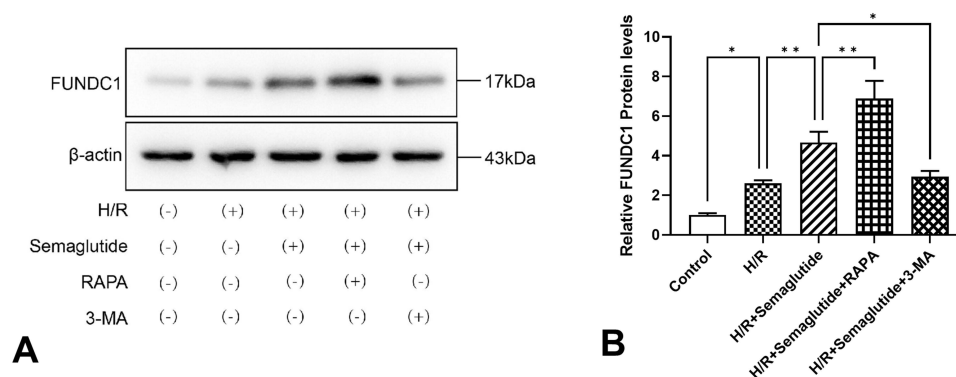


Figure 6 Effect of Semaglutide on the Level of Autophagy-Related Protein FUNDC1 in AC16 Cells Following H/R Treatment. **(A)** Western blot analyses showed the expressions of β -catenin, FUNDC1 in different groups. **(B)** Quantitative data on FUNDC1 in different groups.

Notes: Data are presented as means $\pm$ SD. * $P < 0.05$, ** $P < 0.01$.

Abbreviations: RAPA, rapamycin; 3-MA, 3-methyladenine; H/R, hypoxia/reoxygenation.

Discussion

The therapeutic potential of glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1RAs) has emerged as a transformative approach in managing cardiometabolic diseases, especially in patients with diabetes mellitus complicated by coronary artery disease (CAD). As a class of incretin-based therapies, GLP-1RAs not only improve glycemic control but also offer significant cardiovascular benefits, including reduced major adverse cardiovascular events (MACE) and enhanced cardiac function.¹⁸ Epidemiological studies reveal that many CAD patients also suffer from type 2 diabetes mellitus (T2DM), emphasizing the urgent need for therapies that target both hyperglycemia and cardiovascular risk.¹⁹

Semaglutide, a long-acting GLP-1RA, has attracted attention for its multifaceted actions; previous studies, such as the SUSTAIN-6 trial, showed that semaglutide reduced MACE in T2DM patients with established cardiovascular disease, highlighting its clinical importance.⁹ However, the cellular and molecular mechanisms behind its cardioprotection are not fully understood. This study aimed to elucidate the mechanisms of GLP-1RA-mediated cardioprotection in vitro hypoxia/reoxygenation (H/R) injury model using AC16 cardiomyocytes, a well-recognized human cardiomyocyte cell line. By exploring the roles of oxidative stress, inflammation, apoptosis and autophagy, this research provides new insights into the cardioprotective pathways activated by semaglutide, strengthening its potential as a therapy for diabetes-related cardiovascular complications.

Our study showed that Semaglutide attenuates H/R-Induced Cardiomyocyte Injury via multimodal mechanisms. H/R injury significantly reduced AC16 cell viability (CCK-8 assay) and increased lactate dehydrogenase (LDH) release, indicative of membrane damage. Semaglutide pretreatment restored cell viability and decreased LDH leakage, suggesting its role in preserving cellular integrity. H/R injury induced marked oxidative stress, as evidenced by elevated malondialdehyde (MDA) levels (a lipid peroxidation marker) and reduced superoxide dismutase (SOD) activity. Semaglutide attenuated MDA accumulation and restored SOD activity, indicating its antioxidative capacity. These results corroborate findings that showed that liraglutide reduced reactive oxygen species (ROS) production in rats with experimental diabetes and ischemia-reperfusion injury.²⁰ Additionally, semaglutide suppressed H/R-induced inflammation, decreasing IL-6 and TGF- β 1 levels, respectively. This anti-inflammatory effect is consistent with prior study Semaglutide exerted cardioprotective effects against diabetic heart failure by alleviating cardiac inflammation through Sirt3-dependent RKIP signalling pathway.²¹ Flow cytometry revealed that H/R increased apoptosis rates, which was reversed with semaglutide. Western blot analysis showed that semaglutide downregulated pro-apoptotic proteins (caspase-3, caspase-9, Bax) and upregulated the anti-apoptotic protein Bcl-2. These findings are supported by a study that demonstrated that liraglutide attenuates myocardial ischemia/reperfusion injury through the inhibition of necroptosis by activating GLP-1R/PI3K/Akt pathway.²²

The combination of semaglutide and rapamycin (an mTOR inhibitor) showed enhanced cardioprotection, increasing cell viability and reducing LDH release compared to H/R alone. Notably, adding 3-methyladenine (3MA, an autophagy inhibitor) partially abolished semaglutide's antioxidant, anti-inflammatory, and anti-apoptotic effects, suggesting that autophagy activation contributes to its cardioprotection and indicating a synergistic interaction between GLP-1 signaling and autophagy regulation. In cardiovascular diseases, the activation or inhibition of the autophagy pathway exerts a crucial impact on disease progression and treatment. Mitochondrial selective autophagy, also known as mitophagy, is regulated through two distinct molecular pathways: ubiquitin - dependent and ubiquitin - independent mechanisms. Among them, the canonical ubiquitin - dependent pathway is mainly governed by the PINK1/Parkin axis. Our previous research has revealed that semaglutide can promote mitophagy in myocardial cells suffering from hypoxia/reoxygenation (H/R)-induced injury. This promotion occurs via the ROS/PINK1/Parkin/p62 pathway, ultimately exerting a cardioprotective effect.¹⁷ FUN14 domain - containing protein 1 (FUNDC1) is a receptor protein that is highly expressed on the mitochondrial outer membrane of cardiomyocytes and plays a crucial role in mediating mitophagy. Previous studies have demonstrated that the expression level and phosphorylation status of FUNDC1 are closely related to the onset, progression, and prognosis of various cardiovascular diseases.²³ As a mitochondrial outer membrane receptor, FUNDC1 can bind to LC3, thereby mediating the selective degradation of damaged mitochondria. In the context of this study, Western blot analysis showed that semaglutide significantly upregulated the protein expression of FUNDC1. This finding suggests that semaglutide may activate FUNDC1 - dependent mitophagy. By doing so, it can selectively eliminate damaged mitochondria, which may in turn break the vicious cycle of reactive oxygen species (ROS) - inflammation - apoptosis. While our in vitro findings support semaglutide's potential therapeutic utility, further validation in primary cardiomyocytes and in vivo models is warranted before clinical application.

Limitations and Future Directions

Despite its strengths, this study has limitations. First, the in vitro nature of the AC16 cell model may not fully replicate the complex pathophysiology of myocardial ischemia/reperfusion injury in vivo. Future studies should validate these findings in primary cardiomyocytes and animal models of myocardial infarction. We used AC16 cardiomyocyte cell lines and in the future study, it could be validated in primary cardiomyocytes. Second, while rapamycin and 3MA were used to

modulate autophagy, their specificity may be influenced by off-target effects. Third, the study did not explore the long-term effects of semaglutide on cardiomyocyte function or its impact on other cell types (eg, endothelial cells, fibroblasts) within the cardiac microenvironment. Moreover, although the current research has shed some light on the role of the FUNDC1 pathway, there are still significant limitations, especially considering that only preliminary exploration has been carried out in the non-ubiquitination pathway, and its specific regulatory mechanisms, phosphorylation sites, and interactions with other pathways remain to be further elucidated. Future research should incorporate co-culture systems or in vivo models (eg, myocardial infarction in rodents) to validate these findings. Additionally, mechanistic studies using gene editing or pharmacological inhibitors could further dissect the roles of specific signaling pathways (eg, AMPK, mTOR, NF- κ B) in semaglutide-induced cardioprotection.

Conclusion

In summary, our findings indicate that semaglutide protects AC16 cardiomyocytes from H/R injury via multifaceted mechanisms, including antioxidative, anti-inflammatory, and anti-apoptotic actions, with potential synergism from autophagy modulation. These findings support the hypothesis that GLP-IRAs may exert cardioprotective effects through these pathways in vitro and provide a rationale for further investigation of their therapeutic potential in myocardial ischemia/reperfusion injury using in vivo models.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation.

or across all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Chen W, Li D. Reactive oxygen species (ROS)-responsive nanomedicine for solving ischemia-reperfusion injury. *Front Chem.* 2020;8:732. doi:10.3389/fchem.2020.00732
2. Algoet M, Janssens S, Himmelreich U, et al. Myocardial ischemia-reperfusion injury and the influence of inflammation. *Trends Cardiovasc Med.* 2023;33(6):357–366. doi:10.1016/j.tcm.2022.02.005
3. Li S, Li F, Wang Y, et al. Multiple delivery strategies of nanocarriers for myocardial ischemia-reperfusion injury: current strategies and future prospective. *Drug Deliv.* 2024;31(1):2298514. doi:10.1080/10717544.2023.2298514
4. Lan M, Hou M, Yan J, et al. Cardiomyocyte-targeted anti-inflammatory nanotherapeutics against myocardial ischemia reperfusion (IR) injury. *Nano Res.* 2022;15:9125–9134. doi:10.1007/s12274-022-4553-6
5. Zhao X, Wang M, Wen Z, et al. GLP-1 receptor agonists: beyond their pancreatic effects. *Front Endocrinol.* 2021;12:721135. doi:10.3389/fendo.2021.721135
6. Parab P, Chaudhary P, Mukhtar S, et al. Role of glucagon-like peptide-1 (GLP-1) receptor agonists in cardiovascular risk management in patients with type 2 diabetes mellitus: a systematic review. *Cureus.* 2023;15(9):e45487. doi:10.7759/cureus.45487
7. Lincoff AM, Brown-Frandsen K, Colhoun HM, et al. Semaglutide and cardiovascular outcomes in obesity without diabetes. *N Engl J Med.* 2023;389(24):2221–2232. doi:10.1056/NEJMoa2307563
8. Kristensen SL, Rørth R, Jhund PS, et al. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet Diabetes Endocrinol.* 2019;7(10):776–785. doi:10.1016/S2213-8587(19)30249-9
9. Leiter LA, Bain SC, Bhatt DL, et al. The effect of glucagon-like peptide-1 receptor agonists liraglutide and semaglutide on cardiovascular and renal outcomes across baseline blood pressure categories: analysis of the LEADER and SUSTAIN 6 trials. *Diabetes Obes Metab.* 2020;22(9):1690–1695. doi:10.1111/dom.14079
10. Xiang M, Lu Y, Xin L, et al. Role of oxidative stress in reperfusion following myocardial ischemia and its treatments. *Oxid Med Cell Longev.* 2021;2021:6614009–6614023. doi:10.1155/2021/6614009

11. Zhang Y, Cai Z, Shen Y, et al. Hydrogel-load exosomes derived from dendritic cells improve cardiac function via Treg cells and the polarization of macrophages following myocardial infarction. *J Nanobiotechnology*. 2021b;19:271. doi:10.1186/s12951-021-01016-x
12. Zhang L, Wei Q, Liu X, et al. Exosomal microRNA-98-5p from hypoxic bone marrow mesenchymal stem cells inhibits myocardial ischemia-reperfusion injury by reducing TLR4 and activating the PI3K/Akt signaling pathway. *Int Immunopharmacol*. 2021a;101:107592. doi:10.1016/j.intimp.2021.107592
13. Xie D-M, Zhong Q, Xu X, et al. Alpha lipoic acid-loaded electrospun fibrous patch films protect heart in acute myocardial infarction mice by inhibiting oxidative stress. *Int J Pharm*. 2023;632:122581. doi:10.1016/j.ijpharm.2023.122581
14. Chen Y, Tang W, Huang X, et al. Mitophagy in intracerebral hemorrhage: a new target for therapeutic intervention. *Neural Regen Res*. 2024;19(2):316–323. doi:10.4103/1673-5374.379019
15. Zhu Q, Luo Y, Wen Y, et al. Semaglutide inhibits ischemia/reperfusion-induced cardiomyocyte apoptosis through activating PKG/PKCε/ERK1/2 pathway. *Biochem Biophys Res Commun*. 2023;647:1–8. doi:10.1016/j.bbrc.2023.01.049
16. Liu Y, Li Z, Xu X, et al. Semaglutide attenuates myocardial ischemia-reperfusion injury by inhibiting ferroptosis of cardiomyocytes via activation of PKC-S100A9 axis. *Front Pharmacol*. 2025;16:1529652. doi:10.3389/fphar.2025.1529652
17. Li L, Jin L, Tian Y, Wang J. Semaglutide enhances PINK1/Parkin dependent mitophagy in hypoxia/reoxygenation induced cardiomyocyte injury. *Mol Med Rep*. 2025;31(5):111. PMID: 40017118; PMCID: PMC11884227. doi:10.3892/mmr.2025.13476
18. Neeland IJ, Marso SP, Ayers CR, et al. Effects of liraglutide on visceral and ectopic fat in adults with overweight and obesity at high cardiovascular risk: a randomised, double-blind, placebo-controlled, clinical trial. *Lancet Diabetes Endocrinol*. 2021;9(9):595–605. doi:10.1016/S2213-8587(21)00179-0
19. Pan F, Xu W, Li J, Huang Z, Shu Q. Trends in the disease burden of congenital heart disease in China over the past three decades. *Zhejiang Da Xue Xue Bao Yi Xue Ban*. 2022;51(3):267–277. doi:10.3724/zdxbyxb-2022-0072
20. Hazir S, Coskun G, Sencar L, et al. Effects of liraglutide on the testis of rats with experimental diabetes and ischemia-reperfusion injury. *Biotech Histochem*. 2025;100(6):371–380. doi:10.1080/10520295.2025.2537045
21. Lin K, Wang A, Zhai C, et al. Semaglutide protects against diabetes-associated cardiac inflammation via Sirt3-dependent RKIP pathway. *Br J Pharmacol*. 2025;182(7):1561–1581. doi:10.1111/bph.17327
22. Zhou G, Wu H, Yang J, et al. Liraglutide attenuates myocardial ischemia/reperfusion injury through the inhibition of necroptosis by activating GLP-1R/PI3K/Akt pathway. *Cardiovasc Toxicol*. 2023;23(3–4):161–175. doi:10.1007/s12012-023-09789-3
23. Li G, Li J, Shao R, Zhao J, Chen M. FUNDC1: a promising mitophagy regulator at the mitochondria-associated membrane for cardiovascular diseases. *Front Cell Dev Biol*. 2021;9:788634. doi:10.3389/fcell.2021.788634

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