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PCSK9 Inhibition Protects Against Myocardial Ischemia-Reperfusion Injury in Type 2 Diabetes Rats Via Suppressing Inflammation and Apoptosis

ABSTRACT

Background: Myocardial ischemia-reperfusion (I/R) injury is aggravated in type 2 diabetes mellitus (T2DM) due to metabolic dysfunction, inflammation, and apoptosis. This study investigated the cardioprotective role of alirocumab, a proprotein convertase subtilisin/kexin type 9 (*PCSK9*) inhibitor, compared with atorvastatin.

Methods: Type 2 diabetes mellitus was induced in rats by a high-fat/high-sugar diet plus streptozotocin injection, followed by myocardial I/R through transient ligation of the left anterior descending artery. Rats (n = 6/group) were randomized into Control, non-diabetic I/R, T2DM + I/R, T2DM + I/R + alirocumab, and T2DM + I/R + atorvastatin groups. Alirocumab (10 mg/kg/week, intraperitoneal injection) or atorvastatin (10 mg/kg/day, oral) was administered for 21 days. Outcomes included lipid deposition, myocardial fibrosis, metabolic parameters, inflammatory cytokines, apoptosis, and expression of *PCSK9*, nucleotide-binding oligomerization domain-like receptor protein 3 (*NLRP3*), and Caspase-3, assessed by histology, enzyme-linked immunosorbent assay, terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay, western blotting, and quantitative reverse transcription polymerase chain reaction.

Results: Non-diabetic I/R rats showed increased lipid accumulation, fibrosis, inflammation, and apoptosis compared with controls, while these effects were markedly exacerbated in T2DM + I/R, confirming the amplifying effect of diabetes. Both alirocumab and atorvastatin significantly reduced lipid accumulation, improved hepatic and renal function, lowered free fatty acids and HbA1c, and restored insulin and C-peptide levels ($P < .001$). Treatments also decreased pro-inflammatory cytokines (interleukin-1β [*IL-1β*], interleukin-6 [*IL-6*], tumor necrosis factor-α [*TNF-α*]), inhibited *NLRP3* inflammasome activation, reduced myocardial apoptosis and *caspase-3* activity, and downregulated myocardial *PCSK9*, *NLRP3*, and *caspase-3* expression. Protective effects were comparable between alirocumab and atorvastatin.

Conclusion: Alirocumab and atorvastatin effectively attenuated myocardial I/R injury in T2DM by modulating lipid metabolism, inflammation, and apoptosis. Diabetes substantially intensified I/R-induced cardiac injury, underscoring the importance of metabolic control in cardioprotection.

Keywords: Apoptosis, cardioprotection, inflammation, myocardial ischemia-reperfusion injury, PCSK9 inhibitor, statin, type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from insulin resistance and pancreatic beta-cell dysfunction.[1,2] Type 2 diabetes mellitus is a major global health concern due to its increasing prevalence and association with significant morbidity and mortality, particularly related to cardiovascular complications.[3,4] Cardiovascular complications are the leading cause of mortality in individuals with T2DM, with a higher risk of developing conditions such as myocardial infarction, stroke, heart failure, and peripheral vascular disease compared to individuals without diabetes.[5,6]

ORIGINAL INVESTIGATION

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Epidemiological studies have highlighted the substantial burden of cardiovascular complications in individuals with T2DM.[7] For example, a meta-analysis by Sarwar et al[8] reported that individuals with diabetes have a 2- to 4-fold increased risk of developing cardiovascular disease compared to non-diabetic individuals. Furthermore, the Framingham Heart Study demonstrated that individuals with T2DM have a 2- to 4-fold increased risk of coronary heart disease compared to those without diabetes.[9]

The association between T2DM and cardiovascular complications can be attributed to various factors, including the presence of other risk factors such as hypertension, dyslipidemia, and obesity, as well as the pro-inflammatory and prothrombotic state associated with diabetes.[10-12] In addition, dyslipidemia is a common feature of T2DM characterized by elevated levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides, and decreased levels of high-density lipoprotein cholesterol (HDL-C).[13] Elevated LDL-C levels are particularly concerning as they are a major risk factor for atherosclerosis, the underlying cause of cardiovascular complications in diabetes.[14]

The development of novel therapeutic agents targeting dyslipidemia in individuals with T2DM has shown promise in reducing the risk of cardiovascular complications.[15] One such class of agents is proprotein convertase subtilisin/kexin type 9 (*PCSK9*) inhibitors, which have been shown to significantly lower LDL-C levels and reduce the risk of cardiovascular events in patients with atherosclerotic cardiovascular disease.[16]

Proprotein convertase subtilisin/kexin type 9 is a key regulator of low-density lipoprotein receptor (LDLR) levels and plays a crucial role in lipid metabolism and the development of cardiovascular disease.[17] Proprotein convertase subtilisin/kexin type 9 inhibitors have emerged as a promising therapeutic option for reducing LDL cholesterol levels and lowering the risk of cardiovascular events.[16] By inhibiting PCSK9, these drugs increase the expression of LDLR on the surface of hepatocytes, leading to enhanced clearance of circulating LDL cholesterol from the blood.

HIGHLIGHTS

- Alirocumab, a proprotein convertase subtilisin/kexin type 9 (*PCSK9*) inhibitor, significantly protects against myocardial ischemia-reperfusion injury in type 2 diabetic rats.
- Treatment with alirocumab reduced myocardial lipid accumulation and improved cardiac histology.
- Alirocumab suppressed inflammation by lowering *IL-1β*, *IL-6*, and *TNF-α* levels and inhibiting *NLRP3* inflammasome activation.
- Apoptosis was significantly reduced by alirocumab, as evidenced by decreased TUNEL-positive cells and *caspase-3* expression.
- *PCSK9* inhibition modulated key inflammatory and apoptotic pathways, highlighting its therapeutic potential in diabetic cardiovascular complications.

Despite the well-established benefits of PCSK9 inhibitors in reducing cardiovascular risk, their effects on diabetes-related cardiovascular complications remain less understood. Diabetes is a major risk factor for cardiovascular disease, with individuals experiencing diabetes being at a higher risk of developing adverse cardiovascular events such as myocardial infarction and stroke.[18] However, the specific impact of PCSK9 inhibitors on the incidence and progression of cardiovascular complications in individuals with diabetes is not fully elucidated, highlighting a gap in current research.

Several studies have suggested a potential role for PCSK9 inhibitors in modulating inflammation, oxidative stress, and endothelial dysfunction, which are key mechanisms involved in the pathogenesis of diabetic cardiovascular complications.[19,20] However, the exact mechanisms through which PCSK9 inhibitors may influence the development and progression of cardiovascular complications in individuals with diabetes remain to be elucidated.

In this study, the aim was to investigate the effects of PCSK9 inhibitors on the development of diabetes-related cardiovascular complications. By elucidating the mechanisms underlying the potential benefits of PCSK9 inhibition in individuals with diabetes, the hope is to provide valuable insights into the therapeutic potential of PCSK9 inhibitors in reducing the burden of cardiovascular disease in this high-risk population.

METHODS

Reagents and Animals

All experiments were approved by the Animal Ethics Committee of the hospital. Sprague-Dawley rats were used as experimental animals. The rats were acclimated for 1 week with free access to food and water under standard conditions with a 12-hour light-darkness cycle. Bedding was changed every 3 days, and water bottles and cages were cleaned regularly. Rats were randomly divided into 5 groups: (1) Control, (2) ischemia-reperfusion group without diabetes (I/R), (3) T2DM with ischemia-reperfusion (T2DM + I/R), (4) T2DM + I/R treated with alirocumab (alirocumab), and (5) T2DM + I/R treated with atorvastatin (atorvastatin). Control and I/R groups were fed a standard diet (60% carbohydrates, 10% fat primarily composed of soybean oil, 22% protein, and 8% other components including fiber) ad libitum. The T2DM + I/R, alirocumab, and atorvastatin groups were fed a high-sugar high-fat diet consisting of 50% carbohydrates, 30% fat primarily composed of animal fats, 13% protein, and 7% other components including fiber. The rats were fed for 6-8 weeks, and then streptozotocin (STZ) was administered at a dose of 150 mg/kg to induce diabetes in high-sugar high-fat diet groups. After 3 days, blood glucose levels were measured, and rats with blood glucose levels ≥16.7 mmol/L were considered successfully modeled. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Myocardial Ischemia-Reperfusion Injury Model Establishment in Rats with Type 2 Diabetes Mellitus

After establishing the diabetic rat model, an I/R injury model was established in the diabetic rats. Anesthetized rats were

subjected to left anterior descending coronary artery ligation followed by reperfusion. After 2 hours of reperfusion, the rats were euthanized for further analysis. Successful induction of myocardial I/R injury was confirmed by visual observation of darkening of the distant myocardium, weakened contraction, ST segment (ST) elevation, and T wave heightening in the electrocardiogram. The non-diabetic I/R group underwent the same surgical procedure without STZ induction.

Drug Administration

The modeled diabetic rats were randomly assigned to the T2DM + I/R, alirocumab, or atorvastatin groups. Alirocumab was administered intraperitoneally at 10 mg/kg weekly, while Atorvastatin was given orally at 10 mg/kg/day. Control and I/R groups received equal volumes of distilled water. Treatments lasted for 21 days.

Sample Preparation

At the end of the 21-day intervention period and 24 hours after drug administration, rats from each group were euthanized, and their hearts were quickly harvested, rinsed with phosphate-buffered saline (PBS), and trimmed to remove the base and atrial tissues. The left ventricle was sectioned along the long axis, and the tissue located near the base of the left ventricle was immediately fixed in 4% paraformaldehyde and embedded in paraffin for sectioning (thickness: 4-5 μm).

Staining and Quantification of Lesions in Aortic Artery

The aortic artery was carefully dissected under a stereomicroscope and fixed in 4% paraformaldehyde for 24 hours. The lesions were stained with Oil Red O. The images were obtained by stereomicroscopy and analyzed with Fiji. Considering individual differences in arterial plaque as well as the aortic artery, the percentage of atherosclerotic lesions was determined by dividing the area of red area plaques stained by Oil Red O by the area of the overall aortic artery after microdissection.

Histomorphological Analysis

Histomorphological analysis was performed using hematoxylin and eosin (H&E) staining and Masson staining on myocardial tissue samples. Sections were dewaxed and rehydrated by immersion in eluent and ethanol. For H&E staining, sections were stained with hematoxylin for 5 minutes and eosin for 15 seconds, washed in 1% hydrochloric acid alcohol, dehydrated, and fixed. All sections were observed by microscopy and photographed with ToupView digital software. The distribution and extent of myocardial interstitial fibrosis were observed using the horsetail pine staining method. Analysis was performed using 400 × 3 fields of view. The average percentage of fibrous tissue area to total area was measured and calculated using Image-Pro Plus software.

Immunofluorescence

The TUNEL assay was performed to detect apoptotic cells in the myocardial tissue. Specific steps were followed for the TUNEL assay.

Measurement of Inflammatory Markers

Serum and myocardial levels of inflammatory markers including IL-1β, IL-6, TNF-α, NLRP3, and C-reactive protein (CRP) were assessed using enzyme-linked immunosorbent assay (ELISA). Additionally, ELISA was used to measure PCSK9 levels in the myocardium.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from myocardial tissue of rats using the TRIzol reagent (Tiangen Biotech; China; DP424) and then reverse-transcribed to complementary DNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo, K1622). Quantification was performed using the SYBR Green qPCR Master Mix (Selleck, B21203). Each sample was analyzed in triplicate using the real-time fluorescence quantitative polymerase chain reaction instrument (Applied Biosystems, ABI). The relative expression levels of RNA were calculated using the $2^{-\Delta\Delta Ct}$ method. Primers for PCSK9, NLRP3, Caspase3, or β-actin were used (Table 1).

Western Blot Analysis

Western blotting was performed to detect the protein expression levels of PCSK9, NLRP3, caspase-3, and cleaved caspase-3, using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as a loading control. Myocardial tissues were homogenized and lysed in radio-immunoprecipitation assay (RIPA) buffer containing protease inhibitors. Protein concentration was determined using the bicinchoninic acid (BCA) assay, and equal amounts of protein (30-50 μg) were separated on 10%-12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer onto polyvinylidene fluoride (PVDF) membranes. Membranes were blocked with 5% nonfat milk in Tris-Buffered Saline with Tween-20 (TBST) for 1 hour at room temperature and incubated overnight at 4°C with primary antibodies. The following primary antibodies were used: *cleaved caspase-3* (Rabbit, Proteintech, 25128-1-AP), *caspase-3* (Rabbit, Affinity, AF6311), *NLRP3* (Rabbit, Affinity, DF15549), *PCSK9* (Rabbit, Affinity, DF12687), and *GAPDH* (Mouse, Servicebio, GB12002). After washing, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 hour at room temperature: goat anti-rabbit

Table 1. Primer Sequences Used for Quantitative Reverse Transcription Polymerase Chain Reaction

	Primer Sequences		
Gene	**Forward**	**Reverse**	**Fragment (bp)**
PCSK9	GGGTGAGGGTGTCTATGCTGTCG	GCTGCTGGGCTCTAAGGTTTTCC	179
NLRP3	TTGTGTGAAAAAATGAAGGACCC	CTGAGCAGCACAGTGAAGTAAGG	85
Caspase3	GTATGCTTACTCTACCGCACCCG	AAAGTGGCGTCCAGGGAGAAG	186
β-actin	GTCGTACCACTGGCATTGTG	TCTCAGCTGTGGTGGTGAAG	180

bp, base pairs.

IgG-HRP (Servicebio, GB23303) and goat anti-mouse IgG-HRP (Servicebio, GB23301). Protein bands were visualized using enhanced chemiluminescence reagents and quantified with ImageJ software.

Sample Size Calculation

A priori power analysis was performed using G*Power 3.1 software to determine the minimum number of animals required per group. Based on preliminary pilot experiments and previous reports of myocardial ischemia-reperfusion injury in diabetic rats, the expected effect size (f) for primary outcomes such as myocardial fibrosis and inflammatory cytokines was set at 0.65 (large effect). With an α error probability of 0.05 and a power (1-β) of 0.80, one-way ANOVA indicated that a minimum of 5 animals per group was necessary to detect statistically significant differences. To account for potential dropouts or unsuccessful modeling, 6 rats were included in each group (n = 6).

Statistical Analysis

All data are expressed as mean ± standard error of the mean. Statistical analyses were performed using GraphPad Prism version 10.1.2 (GraphPad Software, San Diego, CA, USA). Differences among groups were analyzed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. A P value < .05 was considered statistically significant.

RESULTS

Effects of Alirocumab and Atorvastatin on Lipid Accumulation and Myocardial Fibrosis

As shown in Figure 1, Oil Red O staining revealed marked lipid accumulation in the aortic artery of the T2DM + I/R group compared with controls. Both alirocumab and atorvastatin significantly reduced lipid deposition, as indicated by a decreased Oil Red O-positive area (P < .0001), with no difference between the 2 treatments. Hematoxylin and eosin staining showed extensive myocardial disorganization and cellular damage in the T2DM + I/R group, which were markedly improved by either treatment, restoring near-normal myocardial structure. Masson's trichrome staining further demonstrated substantial fibrosis in the T2DM + I/R group, while both alirocumab and atorvastatin significantly reduced fibrotic area (P < .0001), with levels approaching those of the controls. These findings indicate that both treatments effectively protect against lipid accumulation and myocardial fibrosis following ischemia-reperfusion injury in T2DM.

Importantly, the non-diabetic I/R group also exhibited significant lipid accumulation and fibrosis compared with controls, though to a lesser extent than the T2DM + I/R group. This observation suggests that diabetes aggravates I/R-induced lipid deposition and fibrotic remodeling.

Given that lipid accumulation and fibrosis were profoundly aggravated in diabetic I/R rats, the next investigation is whether these pathological changes were accompanied by systemic metabolic disturbances.

Effects of Alirocumab and Atorvastatin on Metabolic Parameters

Table 2 summarizes the metabolic and biochemical parameters in the 5 groups (n = 6 each). Compared with controls, the I/R group displayed moderate increases in liver injury markers (alanine aminotransferase [ALT], aspartate

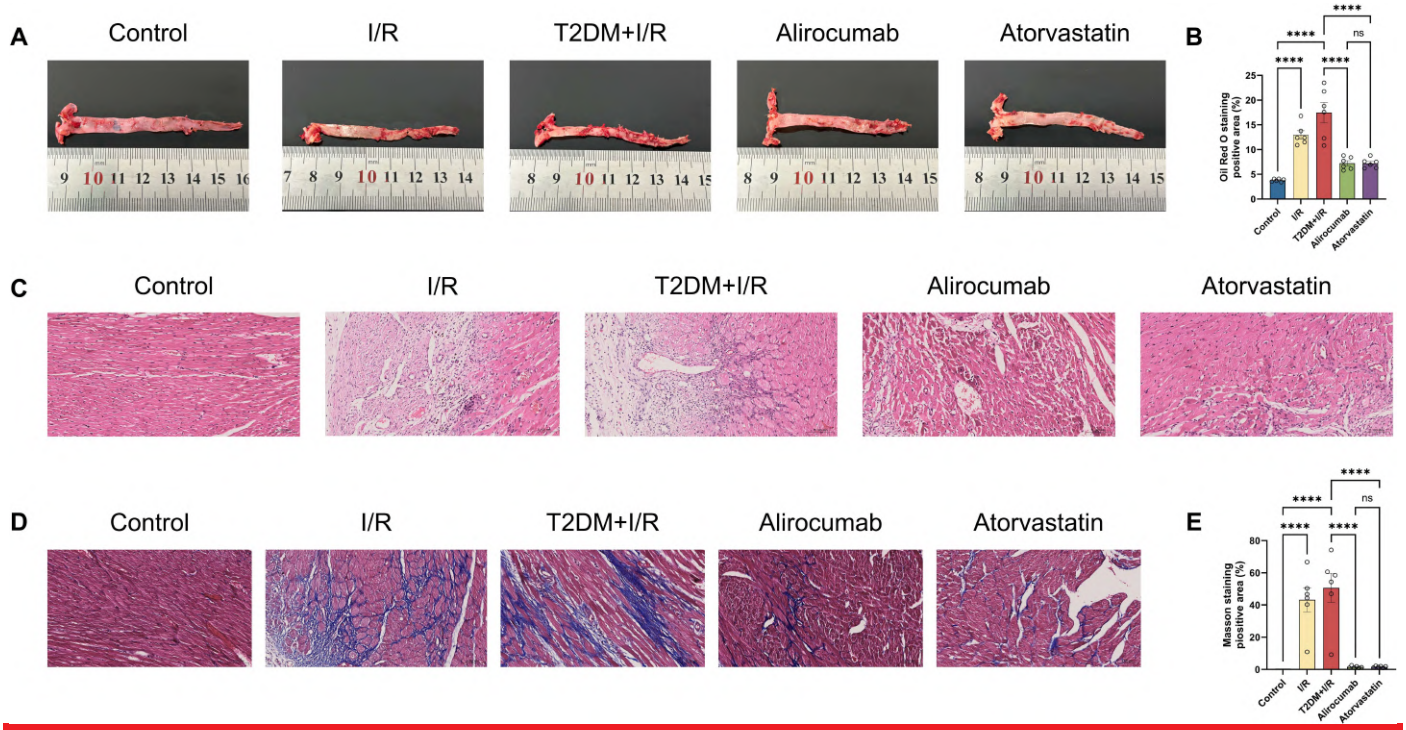

Figure 1. Protective effects of alirocumab and atorvastatin on myocardial ischemia-reperfusion injury in rats (n = 6 per group). (A) Macroscopic images of Oil Red O staining of hearts from Control, I/R, T2DM + I/R, alirocumab, and atorvastatin groups. (B) Quantification of the Oil Red O-positive area (%). (C) Representative hematoxylin and eosin staining images of myocardial tissue. (D) Masson's trichrome staining of myocardial tissue. (E) Quantification of fibrosis area (%). ns, not significant; ****P < .0001.

aminotransferase [AST]), renal markers (urea, creatinine), and dyslipidemia (triacylglycerol [TG], total cholesterol [CHO], LDL), along with reduced HDL. These abnormalities were more pronounced in the T2DM + I/R group, underscoring the amplifying effect of diabetes on I/R-induced organ dysfunction. Both alirocumab and atorvastatin significantly improved these parameters compared with untreated T2DM + I/R rats, lowering ALT, AST, TG, CHO, LDL, urea, and creatinine, while partially restoring HDL. Blood glucose was markedly higher in the T2DM + I/R group than in controls, whereas the I/R group showed intermediate values. Alirocumab and atorvastatin significantly reduced glucose levels compared with untreated T2DM + I/R rats, although values remained above those of controls. Collectively, these results indicate that both therapies alleviate metabolic disturbances and organ injury associated with I/R in the diabetic state. All values in Table 2 are presented as fasting measurements with standard units.

To further delineate these systemic metabolic alterations, specific markers in both serum and myocardial tissue were analyzed.

Effects of Alirocumab and Atorvastatin on Metabolic Markers in Serum and Myocardium

Figure 2 presents serum and myocardial levels of free fatty acids (FFAs), glycosylated hemoglobin (GHb), insulin, and C-peptide. In T2DM + I/R rats, FFAs and GHb levels were markedly elevated ($P < .0001$), reflecting severe metabolic dysregulation, while both treatments significantly reduced these values toward control levels. Serum and myocardial insulin and C-peptide were significantly decreased in T2DM + I/R rats, consistent with impaired pancreatic function. Both alirocumab and atorvastatin significantly restored insulin and C-peptide ($P < .0001$), though levels did not fully normalize. No significant differences were observed between the 2 treatment groups.

The I/R group also showed higher FFAs and GHb and lower insulin and C-peptide than controls, though changes were less pronounced than in T2DM + I/R rats. These results again suggest that diabetes amplifies I/R-induced metabolic disturbances.

Since metabolic dysfunction is closely linked to inflammatory activation, inflammatory cytokines and NLRP3 inflammasome activity were examined.

Effects of Alirocumab and Atorvastatin on Inflammatory Markers and NLRP3 Inflammasome

Figure 3 shows that pro-inflammatory cytokines (*IL-1β*, *IL-6*, *TNF-α*) and *NLRP3* inflammasome activity were significantly increased in both serum and myocardial tissue of T2DM + I/R rats compared with controls ($P < .0001$). Both treatments significantly reduced cytokine levels and *NLRP3* expression ($P < .0001$), confirming strong anti-inflammatory effects. Notably, reduction of *NLRP3* activation is particularly relevant, as this inflammasome plays a pivotal role in regulating myocardial inflammatory responses. There was no significant difference between alirocumab and atorvastatin.

The I/R group also displayed elevated inflammatory cytokines compared with controls, but the levels were consistently lower than those in T2DM + I/R rats, indicating that diabetes worsens the inflammatory response to I/R.

Given the interplay between inflammation and apoptosis in myocardial injury, the extent of cardiomyocyte apoptosis was assessed next.

Effects of Alirocumab and Atorvastatin on Myocardial Apoptosis

Figure 4 demonstrates apoptotic changes in myocardial tissue. The T2DM + I/R group showed a marked increase in apoptosis, with significantly more TUNEL-positive cells compared with controls ($P < .0001$). Both alirocumab and atorvastatin substantially reduced apoptotic cell numbers, restoring them closer to control values, with no significant difference between the 2.

In addition, serum and myocardial *caspase-3* levels were markedly elevated in T2DM + I/R rats, further supporting

Table 2. Comparison of Metabolic Characteristics Among Control, I/R, T2DM + I/R, Alirocumab, and Atorvastatin Rats

	Control (n = 6)	T2DM (n = 6)	I/R (n = 6)	Alirocumab (n = 6)	Atorvastatin (n = 6)
ALT (U/L)	77 ± 5	112 ± 7	157 ± 9	96 ± 6	96 ± 7
AST (U/L)	105 ± 6	148 ± 9	207 ± 13	133 ± 6	134 ± 10
GGT (U/L)	0.38 ± 0.09	0.64 ± 0.10	1.26 ± 0.18	0.79 ± 0.10	0.78 ± 0.09
TBA (umol/L)	1.86 ± 0.21	3.55 ± 0.42	10.09 ± 0.72	5.13 ± 0.47	5.25 ± 0.54
Urea (mmol/L)	1.64 ± 0.31	2.34 ± 0.28	5.58 ± 0.37	3.03 ± 0.29	3.08 ± 0.46
CRE (umol/L)	0.80 ± 0.22	1.85 ± 0.33	6.47 ± 0.53	2.50 ± 0.39	2.47 ± 0.52
UA (mmol/L)	0.16 ± 0.05	0.38 ± 0.06	1.09 ± 0.22	0.56 ± 0.05	0.56 ± 0.06
TG (mmol/L)	0.24 ± 0.05	0.41 ± 0.07	0.87 ± 0.10	0.52 ± 0.07	0.51 ± 0.01
CHO (mmol/L)	2.08 ± 0.14	2.56 ± 0.23	4.87 ± 0.45	3.02 ± 0.26	3.07 ± 0.36
HDL (mmol/L)	0.50 ± 0.05	0.39 ± 0.04	0.16 ± 0.05	0.31 ± 0.03	0.30 ± 0.05
LDL (mmol/L)	0.15 ± 0.04	0.26 ± 0.05	0.57 ± 0.03	0.35 ± 0.05	0.33 ± 0.02
BG (mmol/L)	10.5 ± 0.5	12.8 ± 0.7	20.0 ± 0.8	14.4 ± 0.5	14.4 ± 1.5

Data are expressed as mean ± SEM.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BG, blood glucose; CHO, total cholesterol; CRE, creatinine; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TBA, total bile acid; TG, triacylglycerol; UA, uric acid.

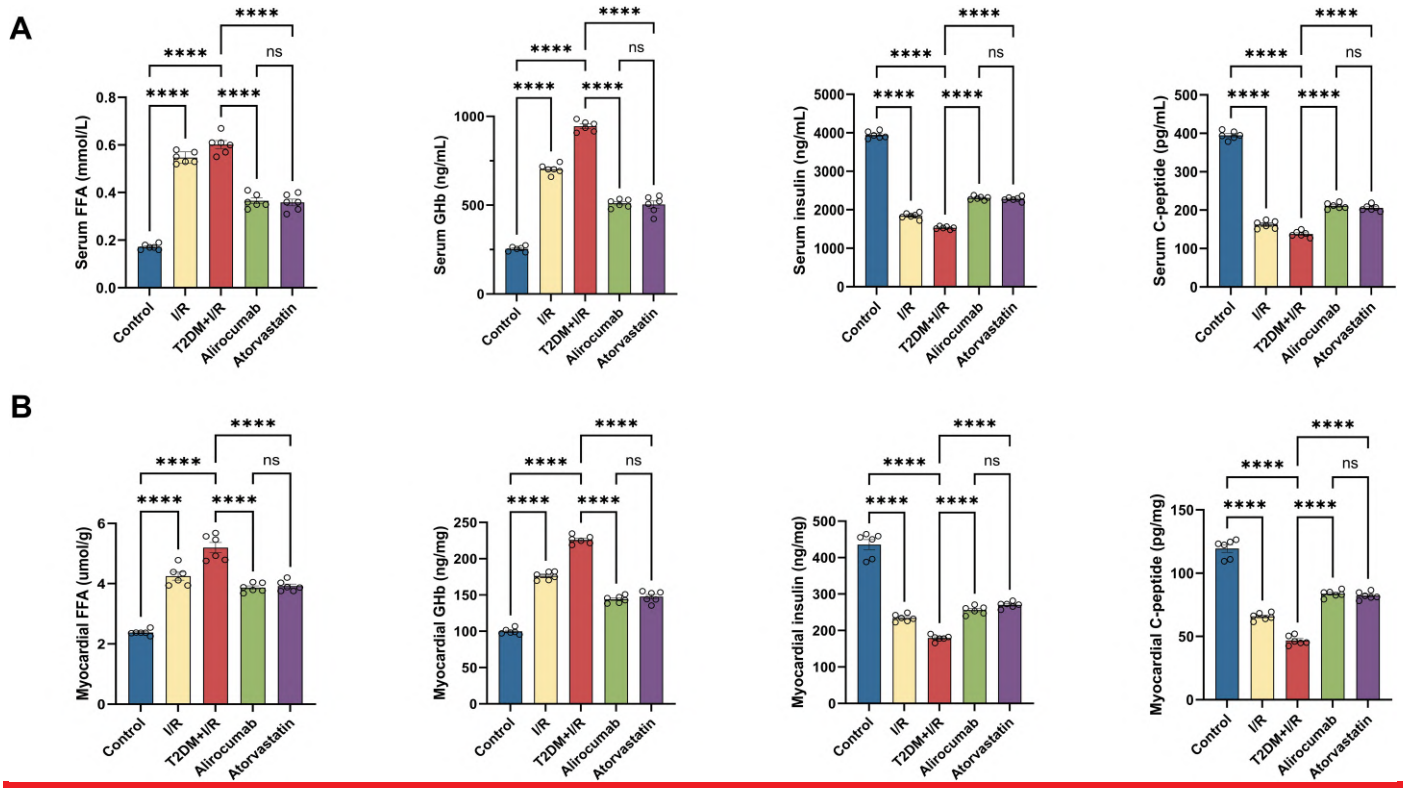

Figure 2. Effects of alirocumab and atorvastatin on serum and myocardial metabolic markers in rats (n = 6 per group). (A) Serum levels of free fatty acids (FFAs), glycosylated hemoglobin (GHb), insulin, and C-peptide in Control, I/R, T2DM + I/R, alirocumab, and atorvastatin groups. (B) Myocardial levels of FFAs, GHb, insulin, and C-peptide. ns, not significant; ****$P < .0001$.

enhanced apoptotic activity. Both treatments significantly reduced *caspase-3* ($P < .0001$), confirming their anti-apoptotic effects. The I/R group also exhibited increased apoptosis and caspase-3 compared with controls, though the magnitude was less than in T2DM + I/R rats. These results highlight the aggravating effect of diabetes on I/R-induced apoptosis.

To further clarify the molecular basis of these pathological processes, the expression of PCSK9, NLRP3, and Caspase-3 was finally examined at both the protein and gene levels.

Effects of Alirocumab and Atorvastatin on Proprotein Convertase Subtilisin/Kexin Type 9, *NLRP3*, and *Caspase-3* Gene Expression

Figure 5 shows the expression of *PCSK9*, *NLRP3*, and *Caspase-3* in myocardial tissues. In T2DM + I/R rats, expression of all 3 genes was significantly upregulated compared with controls ($P < .0001$), reflecting activation of inflammatory (*NLRP3*), apoptotic (*Caspase-3*), and *PCSK9*-mediated pathways. Both alirocumab and atorvastatin significantly downregulated these genes compared with T2DM + I/R ($P < .0001$), with comparable efficacy.

The I/R group also showed increased expression of *PCSK9*, *NLRP3*, and *Caspase-3* relative to controls, but the levels were consistently lower than in T2DM + I/R rats. These findings support the conclusion that diabetes intensifies I/R-induced activation of inflammatory and apoptotic pathways and that both treatments exert cardioprotective effects by suppressing these signals.

DISCUSSION

In this study, it was revealed that both alirocumab and atorvastatin provide significant cardioprotective effects in a rat model of T2DM subjected to myocardial I/R injury. Our major findings indicate that both treatments effectively reduced lipid accumulation, fibrosis, inflammation, and apoptosis in the myocardium. Specifically, alirocumab and atorvastatin improved lipid and glucose metabolism, as reflected by reductions in FFAs, HbA1c, and dyslipidemia. Moreover, both therapies decreased pro-inflammatory cytokines and suppressed *NLRP3* inflammasome activation in serum and myocardial tissues, underscoring their anti-inflammatory potential. Apoptosis was also markedly reduced, as shown by lower levels of TUNEL-positive cells and *caspase-3* expression. At the molecular level, both treatments downregulated the expression of genes involved in inflammation and apoptosis, including *PCSK9*, *NLRP3*, and *caspase-3*. Taken together, these findings suggest that alirocumab and atorvastatin confer significant protection against myocardial I/R injury in T2DM by mitigating metabolic dysfunction, inflammation, and apoptosis.

Our results are largely consistent with prior studies on the cardioprotective effects of *PCSK9* inhibitors and statins. Wu et al[21] demonstrated that *PCSK9* inhibition reduces inflammation and fibrosis in myocardial infarction via the *Notch1* signaling pathway, which aligns with our observation of reduced fibrosis and inflammatory cytokines following alirocumab treatment. Additionally, Huang et al[22] highlighted

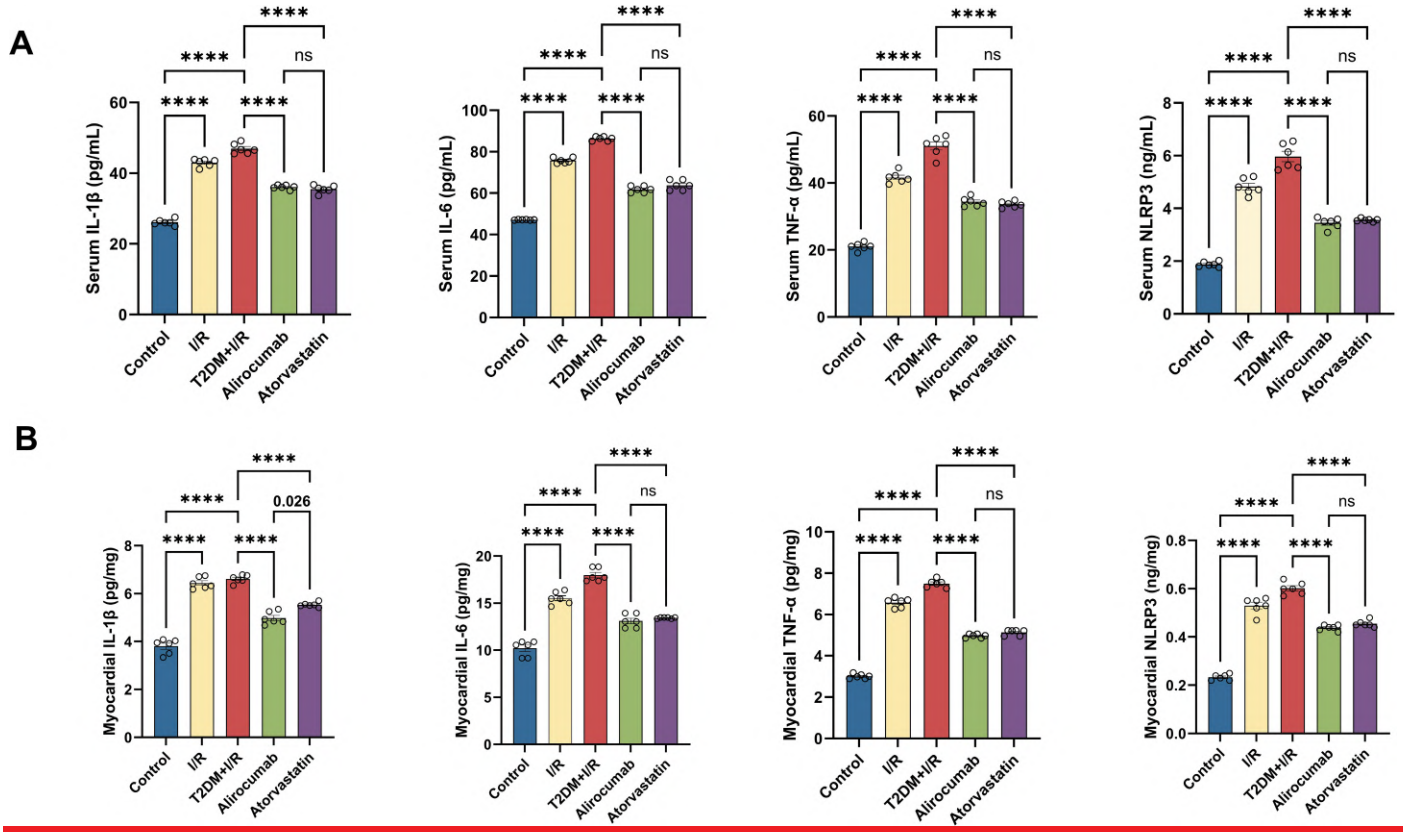

Figure 3. Effects of alirocumab and atorvastatin on pro-inflammatory cytokines and *NLRP3* inflammasome activation (n = 6 per group). (A) Serum levels of *IL-1β*, *IL-6*, *TNF-α*, and *NLRP3* in Control, I/R, T2DM + I/R, alirocumab, and atorvastatin groups. (B) Myocardial levels of *IL-1β*, *IL-6*, *TNF-α*, and *NLRP3*. ns, not significant; ****$P < .0001$.

the protective effects of *PCSK9* inhibition against ischemia-reperfusion injury by modulating autophagy, consistent with our finding that alirocumab alleviates apoptosis and metabolic disturbances. Atorvastatin's ability to reduce inflammation and lipid accumulation in diabetic models has also been widely reported,[23] and our study corroborates these findings by showing improvements in lipid metabolism and inflammatory status in myocardial tissue.

Importantly, our study extends previous research by directly comparing alirocumab and atorvastatin within the same diabetic I/R model. While prior work has primarily focused on either *PCSK9* inhibitors or statins in isolation, our study provides a comparative perspective, demonstrating that both therapies are equally effective in attenuating myocardial injury. Furthermore, novel evidence that both treatments downregulate *PCSK9*, *NLRP3*, and *Caspase-3* gene expression has been provided, linking their protective effects not only to systemic improvements but also to regulation of key molecular pathways.

Growing evidence suggests that *PCSK9* plays an important role in inflammation.[24-26] Elevated *PCSK9* has been positively correlated with circulating CRP levels and shown to be a stronger predictor of cardiovascular disease than LDL-C.[27] Oxidized LDL can upregulate *PCSK9* expression and promote the secretion of *IL-1α*, *IL-6*, and *TNF-α* in a dose-dependent manner.[28] Tang et al[29] reported that *PCSK9* silencing in hyperlipidemic knockout mice reduced TLR pathway activity, thereby decreasing cytokine secretion and atherosclerosis independently of cholesterol levels. Ricci et al[30] further demonstrated that *PCSK9* directly activates *NF-κB* signaling in macrophages. These studies support our findings and suggest that targeting *PCSK9* may suppress inflammatory pathways during myocardial I/R injury.

Another strength of this study is the comprehensive evaluation of gene expression alongside histological and biochemical assessments. Both alirocumab and atorvastatin downregulated *PCSK9*, *NLRP3*, and *Caspase-3* mRNA expression, providing molecular evidence for their cardioprotective actions. This highlights that these therapies not only reduce circulating inflammatory markers but also regulate intracellular signaling pathways central to myocardial injury. In addition, the combined use of TUNEL assays and *caspase-3* activity measurements offered a robust assessment of apoptosis, reinforcing the observed anti-apoptotic effects. This integrative approach strengthens our conclusion that both treatments confer multifaceted protection against I/R injury in T2DM.

The inclusion of a non-diabetic I/R group provided new insights into the interaction between diabetes and ischemia. Our results showed that I/R alone induced lipid accumulation, metabolic disturbances, inflammation, and apoptosis, but these alterations were consistently more severe in the

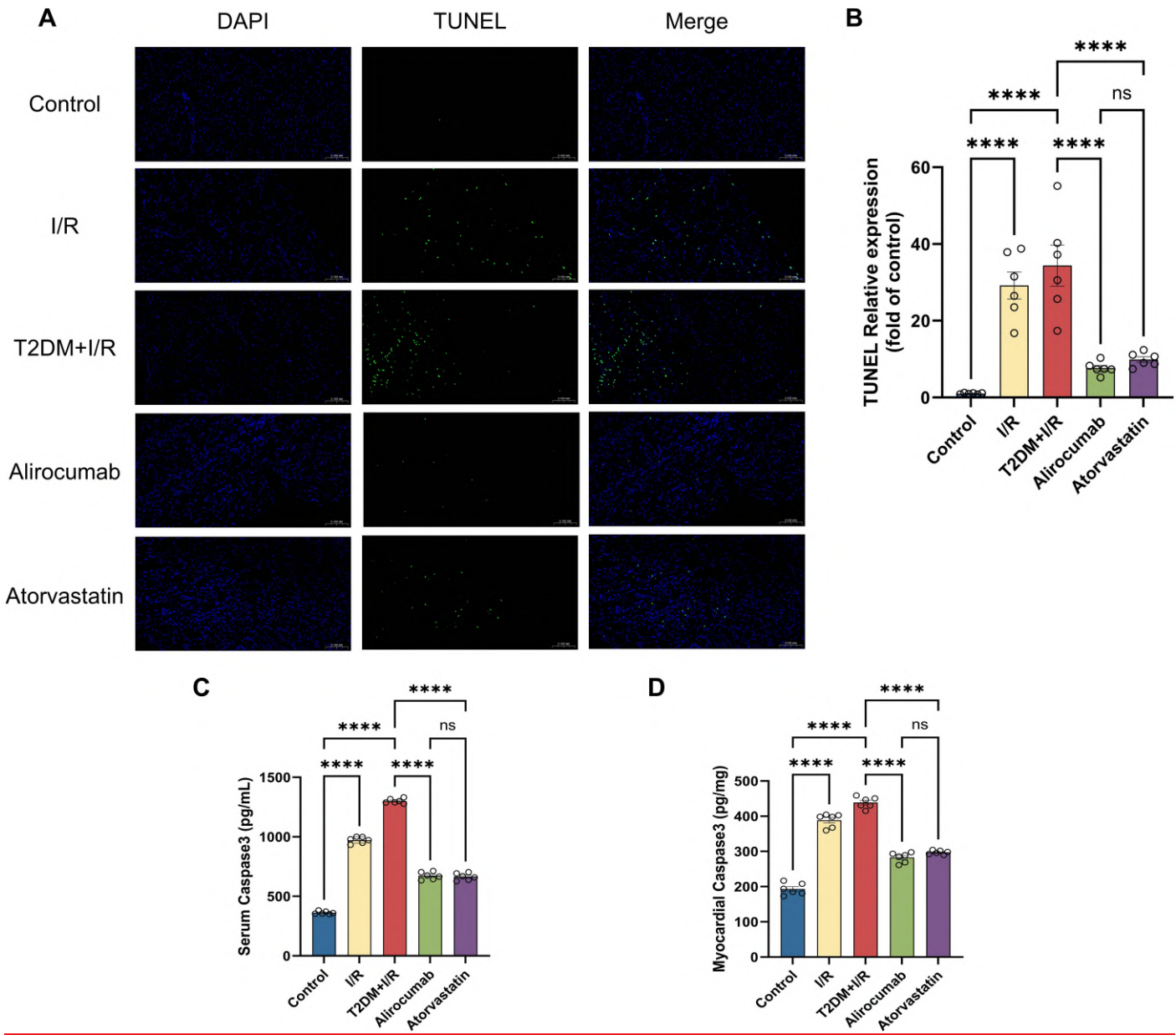

Figure 4. Effects of alirocumab and atorvastatin on myocardial apoptosis and *caspase-3* activity (n = 6 per group). (A) TUNEL assay images (DAPI staining for nuclei and TUNEL for apoptotic cells) in myocardial tissue from Control, I/R, T2DM + I/R, alirocumab, and atorvastatin groups. (B) Quantification of TUNEL-positive cells (fold change relative to control). (C) Serum levels of *caspase-3*. (D) Myocardial levels of *caspase-3*. ns, not significant; ****$P < .0001$. DAPI, 4',6-diamidino-2-phenylindole.

diabetic setting. This confirms that diabetes amplifies myocardial vulnerability to I/R injury, likely through mechanisms involving oxidative stress, mitochondrial dysfunction, and enhanced *NLRP3* activation.[31,32]

Although both treatments were equally effective in our model, their mechanisms may differ. Statins exert pleiotropic actions beyond lipid lowering, including improving endothelial function, reducing oxidative stress, and modulating inflammatory signaling.[33] Proprotein convertase subtilisin/kexin type 9 inhibitors, while primarily targeting cholesterol metabolism, are increasingly recognized for their direct anti-inflammatory and anti-apoptotic effects. Miettinen et al[34] showed that *PCSK9* activates *TLR4* signaling and promotes cytokine release, linking *PCSK9* activity to vascular and myocardial inflammation.[34] Our observation that *PCSK9* expression was elevated in both I/R and T2DM + I/R groups supports this dual role in lipid metabolism and inflammation.

The TUNEL and caspase-3 data further highlight that apoptosis occurs in both non-diabetic and diabetic I/R settings but is significantly amplified by diabetes. This is consistent with Dai et al,[35] who reported that hyperglycemia enhances I/R-induced apoptosis via *caspase-3* activation.[35] These findings highlight the synergistic impact of hyperglycemia and ischemia and underscore the need for therapeutic approaches addressing both metabolic and ischemic stress.

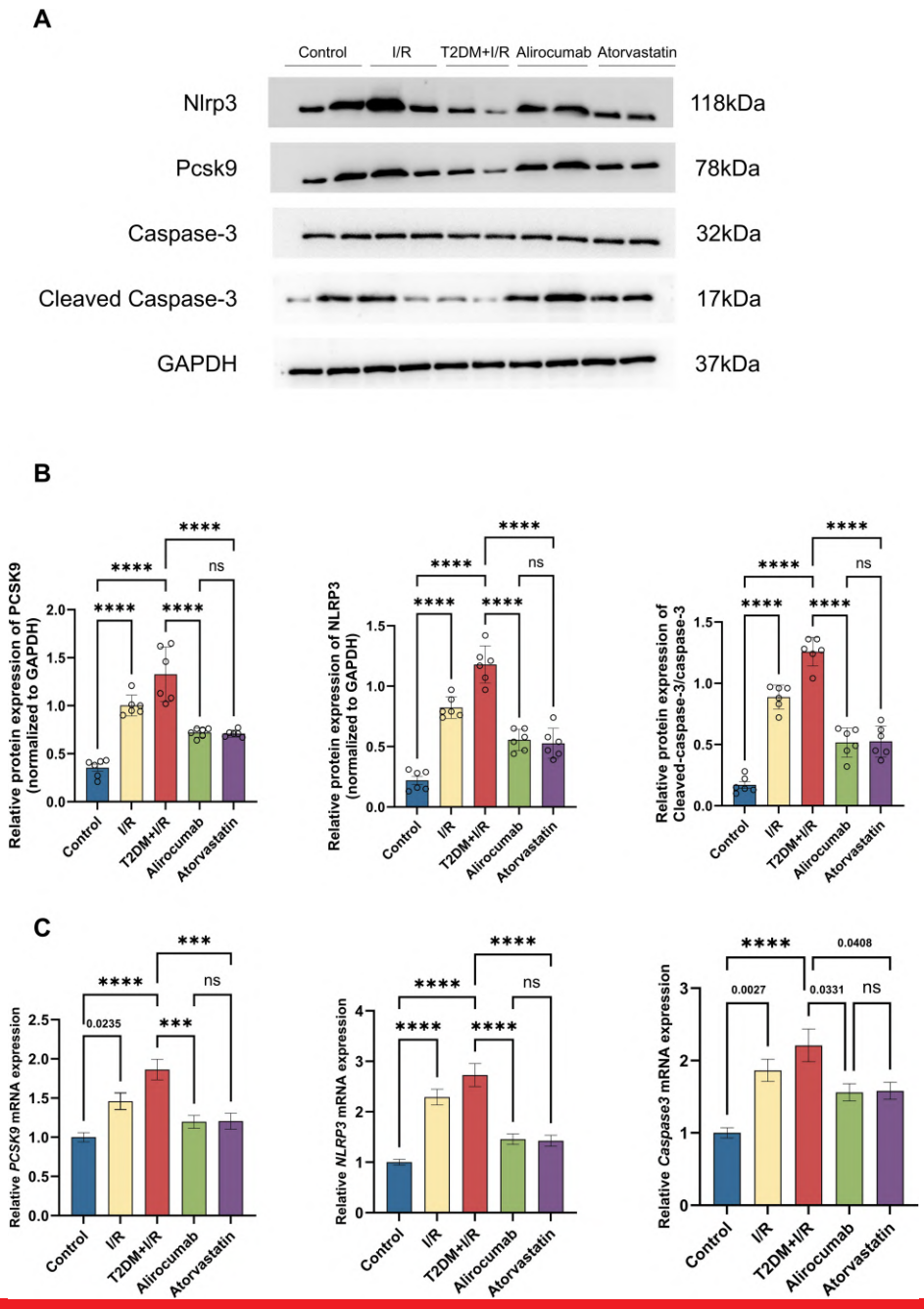

Figure 5. Effects of alirocumab and atorvastatin on mRNA expression of *PCSK9*, *NLRP3*, and *caspase-3* in myocardial tissues (n=6 per group). (A) Western blot analysis of *PCSK9*, *NLRP3*, and *caspase-3* protein levels in control, I/R, T2DM + I/R, alirocumab, and atorvastatin groups. (B) Quantification of Western blot protein expression levels. (C) Quantitative reverse transcription polymerase chain reaction analysis of mRNA expression for *PCSK9*, *NLRP3*, and *caspase-3*. ns, not significant; ***$P < .001$; ****$P < .0001$.

Despite its strengths, this study has several limitations. First, the long-term effects of alirocumab and atorvastatin were not investigated, which limits understanding of sustained efficacy. Second, while *PCSK9*, *NLRP3*, and *Caspase-3* were examined, other critical mechanisms such as autophagy, oxidative stress, and mitochondrial dynamics were not assessed. Finally, the use of a rodent model, while informative, limits direct extrapolation to humans, particularly regarding dosage and treatment response.

Future studies should include larger sample sizes and longer follow-up periods to evaluate sustained cardioprotective effects. Investigating additional pathways such as autophagy, oxidative stress, and mitochondrial function will further clarify underlying mechanisms. Most importantly, clinical trials in human populations are essential to confirm the translational potential of alirocumab and atorvastatin in preventing cardiovascular complications in T2DM, ensuring that preclinical findings can be effectively applied in clinical practice.[36]

CONCLUSION

In conclusion, the present study provides evidence that both statins and *PCSK9* inhibition can attenuate myocardial I/R injury, particularly in the context of T2DM, by reducing inflammation, apoptosis, and metabolic dysfunction. The inclusion of the non-diabetic I/R group highlights that diabetes amplifies I/R-induced damage, providing mechanistic insight into the heightened cardiovascular risk in diabetic patients. Together, these findings underscore the translational potential of *PCSK9* inhibition and statin therapy as complementary strategies in managing diabetes-related cardiovascular complications.

Ethics Committee Approval: This study was approved by the Animal Ethics Committee of Baotou Central Hospital (Approval No.: KYLL2024-099; Date: 2024-08).

Informed Consent: This study did not involve human subjects; therefore, informed consent is not applicable.

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Declaration of Interests: The authors have no conflicts of interest to declare.

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