

Inhibition of AIM2 Inflammasome-Mediated Macrophage Polarization Underlies the Cardioprotective Effects of Ginsenoside Rg1 in Myocardial Ischemia/Reperfusion Injury

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Received: 12 September 2024; Revised: 08 December 2024; Accepted: 10 December 2024

ABSTRACT

Myocardial ischemia/reperfusion (MI/R) injury represents a primary etiology of mortality on a global scale, presenting a critical challenge to cardiovascular well-being. Prior research indicates that Ginsenoside Rg1 exerts suppressive effects on inflammatory cascade activation, oxidative stress, and myocardial damage, implying its potential utility as a therapeutic intervention for MI/R injury. Nevertheless, the precise underlying molecular mechanisms require more comprehensive investigation. An *in vivo* MI/R model was established in Sprague-Dawley rats via ligation of the left anterior descending coronary artery. Myocarditis, macrophage polarization, and fibrotic changes were assessed by evaluating the organ index, electrocardiographic alterations, myocardial infarct size, histopathological variations, and the expression profiles of cardiac injury markers and pro-inflammatory mediators. Additionally, rat bone marrow-derived macrophages (BMDMs) were utilized to explore the *in vitro* regulatory impacts of Rg1 on absent in melanoma 2 (AIM2) inflammasome activation and macrophage phenotypic polarization. The administration of Rg1 demonstrated dose-dependent cardioprotective properties and substantially mitigated MI/R-induced damage. Rg1 markedly suppressed myocardial inflammation and impeded M1 macrophage polarization during the course of MI/R injury. Moreover, Rg1 considerably decreased cardiac fibrosis secondary to MI/R insult. This anti-fibrotic activity may play a pivotal role in maintaining myocardial structural integrity and ventricular function post-ischemia. Concurrently, Rg1 efficiently obstructed the activation of the AIM2 inflammasome *in vitro*, emphasizing its capability to serve as a critical modulator of inflammatory signaling pathways. The present study clarifies the diverse mechanisms mediating the cardioprotective efficacy of Rg1, specifically demonstrating its capacity to alleviate inflammatory responses, modulate macrophage polarization states, and suppress fibrotic progression.

Keywords: AIM2 inflammasome, Cardiac fibrosis, Ginsenoside Rg1, Myocardial ischemia-reperfusion injury (MIRI), Macrophage polarization

How to Cite This Article: Eriksen K, Berg S, Dahl M. Inhibition of AIM2 Inflammasome-Mediated Macrophage Polarization Underlies the Cardioprotective Effects of Ginsenoside Rg1 in Myocardial Ischemia/Reperfusion Injury. *Spec J Pharmacogn Phytochem Biotechnol*. 2024;4(2):116-31. <https://doi.org/10.51847/mLGSN7ORAw>

Introduction

Coronary artery disease (CAD) represents a critical global healthcare challenge characterized by elevated rates of morbidity and mortality. The primary pathological feature involves myocardial damage induced by ischemia-reperfusion processes [1]. Consequently, myocardial ischemia/reperfusion (MI/R) injury has garnered substantial academic interest due to its clinical prominence and accompanying therapeutic difficulties. These injuries occur frequently following acute myocardial infarction events [2]. Ischemia within the myocardium is characterized by the degradation of cardiac cells resulting from prolonged oxygen deprivation, which sequentially impairs the pumping capability of the heart. Although reperfusion following an ischemic episode typically restores the

structural integrity of the damaged tissue and enhances cardiac performance, the sudden re-establishment of blood flow can paradoxically accelerate the death of compromised cardiomyocytes. This occurs via the induction of complex cellular cascades, including the infiltration of inflammatory cells [3]. Accordingly, inflammatory reactions serve as a pivotal mechanism driving cardiomyocyte damage.

The interplay among MI/R, inflammatory corpuscle activation, and macrophage polarization constitutes an active area of contemporary research. Suppressing the inflammatory cascade represents a primary strategy for preventing and managing MI/R injury, given that myocardial inflammation serves as a dominant pathological hallmark of this condition [4]. Within the MI/R paradigm, the AIM2-ASC-caspase 1 inflammasome is activated by DNA derived from necrotic cells, which subsequently triggers an increased secretion of inflammatory mediators, accelerates cardiac fibrosis, and worsens cardiomyocyte degeneration [5]. The protein ubiquitination pathway acts as an essential regulatory mechanism for inflammasome activation; specifically, the K27-linked ubiquitination of absent in melanoma 2 (AIM2) facilitates its activation. This molecular event stabilizes AIM2, thereby promoting inflammasome assembly, ASC speck formation, and sustained activation of caspase-1 [6, 7].

Furthermore, macrophages occupy a central role in these processes through their phenotypic polarization into either pro-inflammatory M1 or anti-inflammatory M2 states. Inflammatory signaling cascades prompt inflammasome activation, which can induce an overproduction of AIM2 in macrophages, thereby amplifying inflammasome propagation and driving a phenotypic shift from the M2 to the M1 state. Notably, AIM2 functions as a specific regulator of M1 macrophage polarization [8]. Hence, targeting the activation of the AIM2 inflammasome and preventing the M1 polarization of macrophages may mitigate MI/R-induced myocardial damage. To date, however, sparse research has explored the therapeutic modulation of MI/R injury through this particular pathway.

Ginsenoside Rg1 is a triterpenoid saponin extracted from the roots and rhizomes of *Panax ginseng* Meyer [9]. This compound exhibits a broad spectrum of pharmacological properties, including antidepressant activities, the mitigation of acute kidney injury, and the alleviation of cardiac dysfunction [10-12]. Additionally, prior evidence [13] indicates that Rg1 prevents the chemotherapy-induced phenotypic shift of microglia from an M2 to an M1 state. Earlier investigations have also demonstrated that Rg1 reduces cardiomyocyte apoptosis and inflammatory responses through distinct molecular pathways [14]. Nevertheless, it remains undetermined whether Rg1 exerts an effect on AIM2 inflammasome activation within cardiomyocytes and on the M1 polarization of macrophages. Ginsenoside Rg1 therefore represents a promising therapeutic agent for alleviating MI/R injury and enhancing cardiac function. The present study demonstrates that Rg1 attenuates myocardial inflammation and further evaluates its cardioprotective efficacy and potential mechanisms in the context of MI/R injury via the AIM2 signaling pathway.

Materials and Methods

Drugs and reagents

Rg1 (purity $\geq 98\%$) was obtained from Shanghai Yuanye Bio-Technology CO., LTD (Shanghai, China). Diltiazem, utilized as the positive control agent, was sourced from Sigma-Aldrich.

Animals and MI/R model establishment

Male Sprague-Dawley (SD) rats, weighing 250–300 g and aged 8–10 weeks, were acquired from Sibeifu Biotechnology Co., Ltd. (Beijing, China). The animals were housed collectively in a climate-controlled environment maintained on a 12:12 h light-dark cycle, with unrestricted access to water and a standard rodent diet. The surgical establishment of the MI/R model adhered to previously documented protocols [15]. Rats assigned to the sham surgical cohort underwent an identical operative procedure, with the exception that the coronary ligature remained untied. Following a 40-min period of ischemia, reperfusion was initiated by gently pulling the slipknot sutures in opposing directions, and this reperfusion phase was maintained for a duration of 7 days. All experimental procedures involving animals were conducted in strict compliance with the guidelines authorized by the Animal Experimentation Ethics Committee of Nanjing University of Chinese Medicine.

Grouping and treatment

Following a 1-week period of environmental acclimation, the animals were subjected to surgical modeling and subsequent pharmacological interventions. The rats were randomly allocated into five experimental cohorts ($n =$

12 per group): the sham group (subjected to sham surgery), the model group (subjected to MI/R), the Rg1 treatment groups (administered at dosages of 10 and 20 mg/kg) [16], and the diltiazem treatment group (administered at a dosage of 20 mg/kg) [17]. Dose selection was guided by parameters established in previous literature. The sham and MI/R cohorts received vehicle administration via injection of distilled water (1 mL/100 g). For the active intervention arms, Rg1 and diltiazem were administered initially within the first 2 h of coronary ligation and subsequently once daily throughout the post-ligation phase. An initial subset of animals ($n = 3$) was sacrificed 3 days post-modeling/administration to perform TTC staining, while the remaining subset ($n = 9$) was sacrificed 7 days post-modeling/administration to facilitate HE staining, serum biochemical assays, Western blot analyses, and tissue immunofluorescence staining, among other evaluations [18]. A schematic representation outlining the *in vivo* experimental design is depicted in **Figure 1a**.

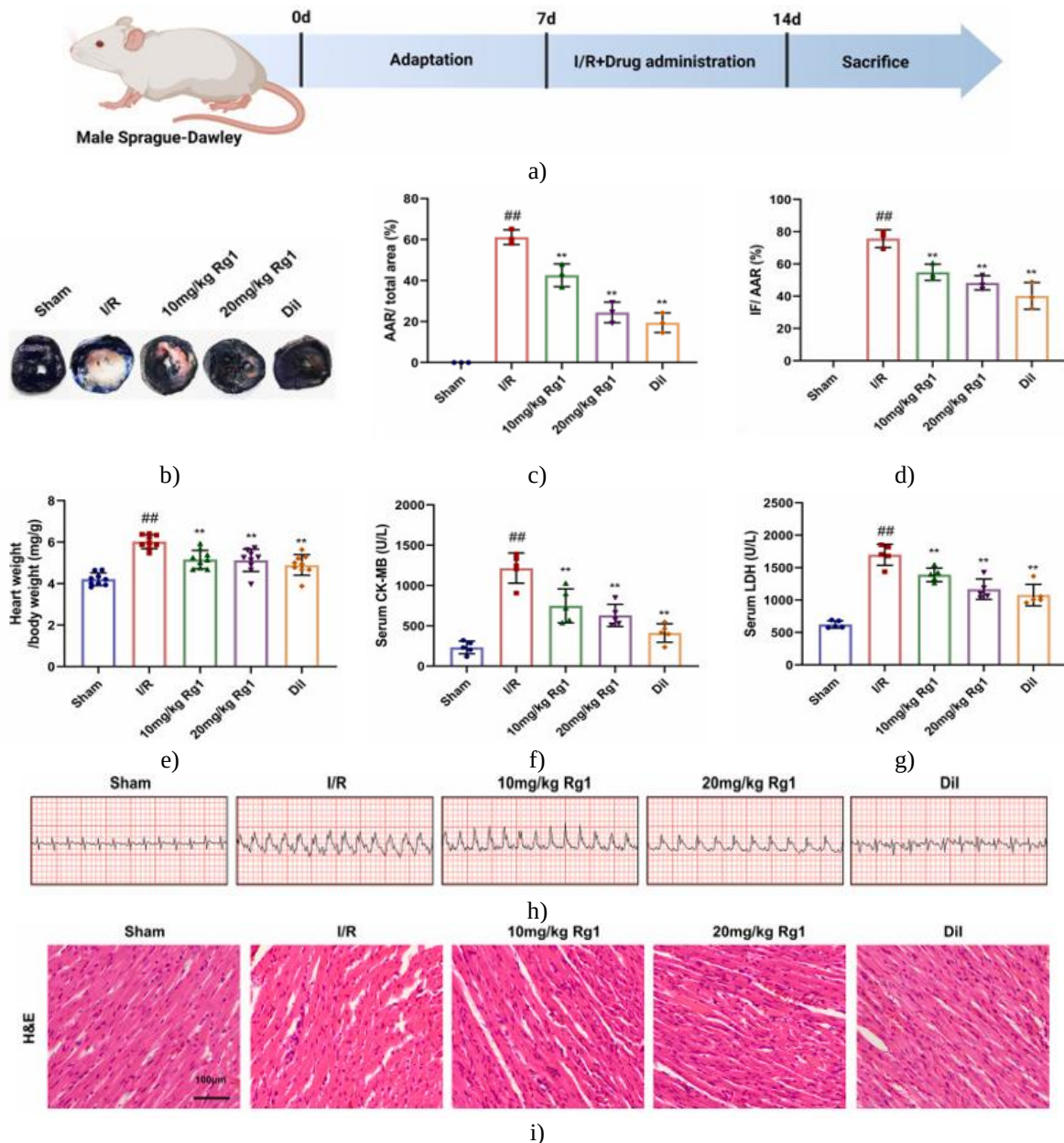


Figure 1. Rg1 prevents MI/R-induced damage in a dose-dependent manner. (a) Schematic overview of the *in vivo* experimental timeline and study design; (b) TTC-Evans blue dual staining of harvested rat myocardial tissues; (c) Quantitative ratio of the area at risk to the total ventricular area (AAR/total area); (d) Quantitative ratio of the infarcted zone to the area at risk (IF/AAR); (e) Calculated heart weight index; (f–g) Serum concentrations of the cardiac biomarkers LDH and CK-MB; (h) Representative electrocardiographic tracings; (i) Histopathological evaluation via HE staining of rat myocardial sections (scale bar = 100 μm). Data are

expressed as mean values $\pm$ standard deviation (SD); ###P < 0.01 relative to the Sham cohort, **P < 0.01 relative to the I/R model cohort.

Assessment of myocardial weight

To determine the heart weight index (expressed in mg/g), the overall cardiac mass of the second rat cohort (n = 9) was quantified across all experimental arms utilizing a high-precision analytical balance manufactured by Shimadzu (Japan).

Quantification of ventricular infarction size

Following the induction of MI/R damage, the macroscopic extent of tissue necrosis was mapped via a dual triphenyltetrazolium chloride (TTC) and Evans blue perfusion technique, mirroring a previously validated protocol [19]. This staining paradigm differentiates three distinct zones: salvaged but threatened myocardium (the area at risk [AAR], appearing deep red), infarcted tissue (IF, appearing white), and unaffected, normally perfused segments (appearing blue). Digital images of these myocardial slices were subsequently processed and statistically evaluated using the ImageJ software package.

Electrocardiographic (ECG) monitoring

At the conclusion of the 7-day post-operative monitoring and pharmacological intervention phase, the rats were somatically sedated via an intraperitoneal injection of 4% sodium pentobarbital. This immobilization allowed for the recording and systematic analysis of updates in their electrical cardiac profiles.

Serum biomarker quantitation

Whole blood collected via ophthalmic venous puncture was kept at 4 °C overnight to facilitate clot retraction, followed by a 15-minute centrifugation step at 1000×g to isolate the serum fraction. The enzymatic activities of crucial cardiac injury indicators—specifically lactate dehydrogenase (LDH) and the creatine kinase isoenzyme MB (CK-MB)—were subsequently measured using commercially available diagnostic kits obtained from Beyotime (China).

Histopathological profiling

Structural anomalies and remodeling within the myocardial tissue framework were evaluated using hematoxylin and eosin (HE) alongside Masson's trichrome staining, adhering to established experimental workflows [20]. Formaldehyde-stabilized, paraffinembedded cardiac tissues were sectioned thin and stained using hematoxylin for 5 min and eosin for 2 min, or otherwise processed with a specialized Masson trichrome staining system (Solarbio, China) to highlight collagen deposition.

Primary cell isolation and in vitro interventions

Primary bone marrow-derived macrophages (BMDMs) were harvested from the medullary cavities of rat femurs and tibias, then differentiated over 7 days in RPMI 1640 medium enriched with 10% fetal bovine serum and 10% L929 cell-conditioned supernatant prior to downstream assays [21]. The cells were distributed into three primary in vitro arms: a Control group, an untreated Model group (challenged with necrotic cell DNA), and experimental arms receiving Rg1 at varying concentrations (1, 2, and 4 μ M) [22]. Macrophages across all groups were first primed for 4 h using 1 g/mL LPS in RPMI 1640 medium containing 0.5% fetal bovine serum. To induce inflammasome activation, the cells were then incubated at 37 °C for 3 h with necrotic cellular DNA that had been pre-labeled with Sytox Orange dye at a density of 800 ng DNA per 2×10^5 cells. Critically, the DNA strands introduced into the control group were enzymatically degraded beforehand using DNase I, while the DNA challenges for the remaining groups were kept completely intact [23].

Tissue immunofluorescence processing

Following routine deparaffinization and sequential rehydration steps, the embedded myocardial sections were exposed overnight at 4 °C to primary antibodies targeting F4/80 (1:1,000, Thermo Fisher, Waltham, MA, USA) and AIM2 (1:1,000; GeneTex, USA). Detection was achieved through subsequent incubation with species-matched, fluorophore-tagged secondary antibodies, including Goat Anti-Rabbit, Anti-rat, and Donkey Anti-Goat IgG H&L (1:5,000, Abcam, Cambridge, MA, USA). Cellular nuclei were visualized via DAPI counterstaining

before the slides were permanently mounted under neutral resin. ImageJ software was employed to calculate the fluorescence intensity of each targeted protein.

Immunoblotting analysis

Total protein was extracted by homogenizing harvested cells and left ventricular tissues in an ice-cold RIPA cell lysis formulation, with final protein concentrations determined via a standard Bradford BCA assay kit (Beyotime, China). Equal protein masses (30 mg per sample) were resolved via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto 0.2 µm polyvinylidene fluoride substrates (Millipore, USA). These membranes were then blocked in an appropriate buffer solution for 1 h, probed overnight at 4 °C with primary antibodies, and subsequently incubated with secondary antibodies for an additional hour at 25 °C. After multiple washes with TBST, the target protein bands were visualized using a Tanon gel documentation system (Bio-Rad, China) and their respective optical densities were analyzed via ImageJ.

Isolation of necrotic cell DNA

To generate standard necrotic remnants, cultured rat ventricular cardiomyocytes were exposed to 500 mM hydrogen peroxide at 37 °C for 3 h. These dying cells were subsequently stained with Sytox Orange dye, washed thoroughly, and collected via centrifugation at 20,000×g for 5 min. Mechanical cell disruption was carried out using a high-throughput tissue homogenizer (Scientz, China), followed by the addition of a specialized DNase inactivation reagent to preserve the extracted necrotic DNA strands.

Sytox orange phagocytosis and ASC speck assays

For the intracellular tracking experiments, cellular internalization of the Sytox Orange-tagged necrotic DNA was monitored under white light microscopy, where the labeled material produced a distinct red signal. In parallel experiments assessing inflammasome assembly, the physical clustering of ASC into macromolecular specks was captured using immunofluorescence microscopy as an indicator of activation; notably, the necrotic DNA applied during this phase was kept free of any SYTOX Orange dye. Following a 24-h incubation phase under their respective drug and DNA treatment conditions, the cells were fixed and probed with anti-ASC primary and secondary antibodies (Abcam, Cambridge, MA, USA). After nuclear counterstaining with DAPI and the application of an anti-quenching medium, the mounted specimens were evaluated via fluorescence microscopy.

Flow cytometric phenotyping

Macrophage differentiation states were categorized into M1 pro-inflammatory profiles (CD11b⁺CD16/32⁺) or M2 anti-inflammatory profiles (CD11b⁺CD206⁺) via flow cytometry, utilizing unlabeled necrotic cell DNA. Following a 24-h treatment window in the presence or absence of compounds and necrotic cell debris, cells were labeled using a FITC-conjugated anti-CD11b monoclonal antibody (BioLegend, San Diego, CA, USA) alongside a primary antibody against CD16/32 (Thermo Fisher, Waltham, MA, USA), the latter tracked via an APC-linked secondary antibody (Thermo Fisher, Waltham, MA, USA). Alternatively, cells were incubated with a primary anti-CD11b antibody (BioLegend, San Diego, CA, USA) and a primary anti-CD206 antibody (Thermo Fisher, Waltham, MA, USA), followed by a PE-tagged secondary antibody (Thermo Fisher, Waltham, MA, USA). Quantitative shifts in these cell populations were evaluated on a BD FACSCanto II flow cytometer (USA).

Quantitative real-time PCR (qRT-PCR)

Total cellular RNA isolation was executed using Trizol Reagent ® (Sigma, USA), followed by reverse transcription using a commercial qPCR SYBR Green Master Mix (High Rox Plus) quantitative PCR Kit (Yeasten, China). The relative abundance of mRNAs encoding interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), inducible nitric oxide synthase (iNOS), and arginase-1 (Arg-1) was monitored via real-time amplification. The definitive primer pairs are organized in **Table 1**.

Table 1. Nucleotide sequence properties of the primers utilized for quantitative real-time PCR.

Primer	Forward (5'-3')	Reverse(5'-3')
TNF-α	GGCTTTCGGAAGTCACTGGA	CCCGTAGGGCGATTACAGTC
IL-1β	TTGAGTCTGCACAGTTCCCC	GTCCTGGGGAAGGCATTAGG
IL-10	CATTCCATCCGGGGTGACAA	GTAGATGCCGGGTGGTTCAA

iNOS	TGGAGCGAGTTGTGGATTGTC	TATCCGTCTCGTCCGTGGCA
Arg-1	CCAGAAGAATGGAAGAGTCAGTGT	GCAGATATGCAGGGAGTCACC
GAPDH	GCATCTTCTTGTGCAGTGCC	GATGGTGATGGGTTTCCCGT

Statistical processing

All experimental measurements are summarized as mean values $\pm$ standard deviation (SD). Group variances were determined using a one-way analysis of variance (ANOVA), with subsequent inter-group comparisons resolved via Dunnett's post-hoc multiple reflection test inside GraphPad Prism 8. P-values less than 0.05 were accepted as indicating statistical significance.

Results and Discussion

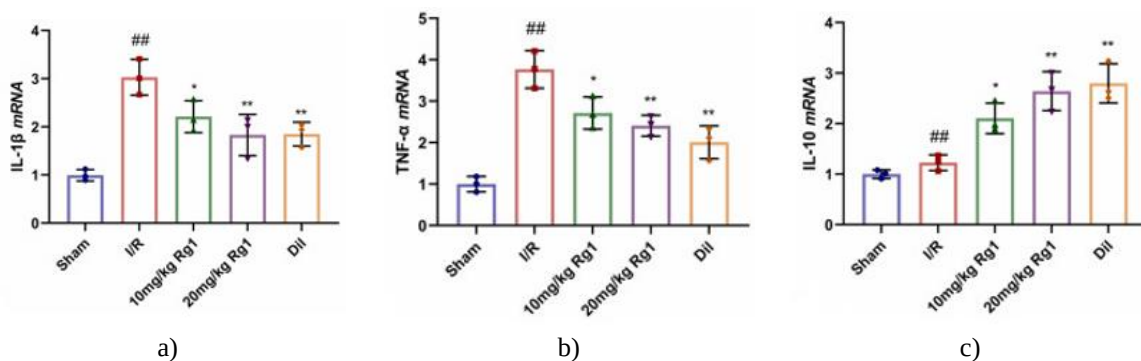
Rg1 mitigates MI/R injury in a dose-dependent manner

To evaluate the therapeutic potential of Rg1 against myocardial ischemia/reperfusion (MI/R) insults, a rat model was established. Dual histological assessment via triphenyltetrazolium chloride (TTC) and Evans blue staining demonstrated that the unmanaged MI/R cohort manifested substantially enlarged areas at risk (AAR) and expanded infarct zones (IF) within the myocardial tissue. Notably, these pathological expansions were significantly curtailed post-intervention. The group allocated the higher concentration of Rg1 exhibited a more pronounced contraction of the infarcted tissue volume than its lower-dose counterpart, verifying that Rg1 limits the scope of myocardial infarction via a dose-dependent mechanism (**Figures 1b and 1d**). Furthermore, this pharmacological approach successfully constrained the elevation of the heart weight index secondary to ischemic reperfusion injury (**Figure 1e**).

Simultaneously, evaluating two highly sensitive serum biomarkers for cardiac damage indicated that the enzymatic activities of both lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) surged markedly in the MI/R model group (**Figures 1f and 1g**). Electrocardiographic monitoring corroborated these findings, revealing a striking elevation of the ST-segment in the untreated ischemic cohort (**Figure 1h**). Histopathological profiling through hematoxylin and eosin (HE) staining showcased preserved cellular architecture, densely organized cardiomyocytes, and linear myofibrillar arrangements within the sham-operated animals. Conversely, the MI/R group exhibited cellular lysis, necrotic fragmentation of cardiomyocytes, disrupted tissue geometry, and robust infiltration of inflammatory leukocytes (**Figure 1i**). This cascade of degenerative features was effectively reversed following treatment with either Rg1 or Diltiazem; notably, the therapeutic efficacy observed at an Rg1 concentration of 20 mg/kg surpassed that achieved at 10 mg/kg.

Rg1 attenuates MI/R-induced myocardial inflammation

To clarify whether the cardioprotective properties of Rg1 stem from the suppression of inflammatory cascades, the transcript abundance of three pivotal inflammatory mediators was quantified within cardiac tissues. In rats subjected to MI/R, the transcription levels of IL-1 β and TNF- α mRNA experienced a massive up-regulation, whereas fluctuations in IL-10 expression did not reach statistical significance. Following administration of Rg1 or diltiazem, these elevated transcripts were suppressed, with the exclusion of IL-10 mRNA, which remained largely unaltered (**Figures 2a and 2c**).



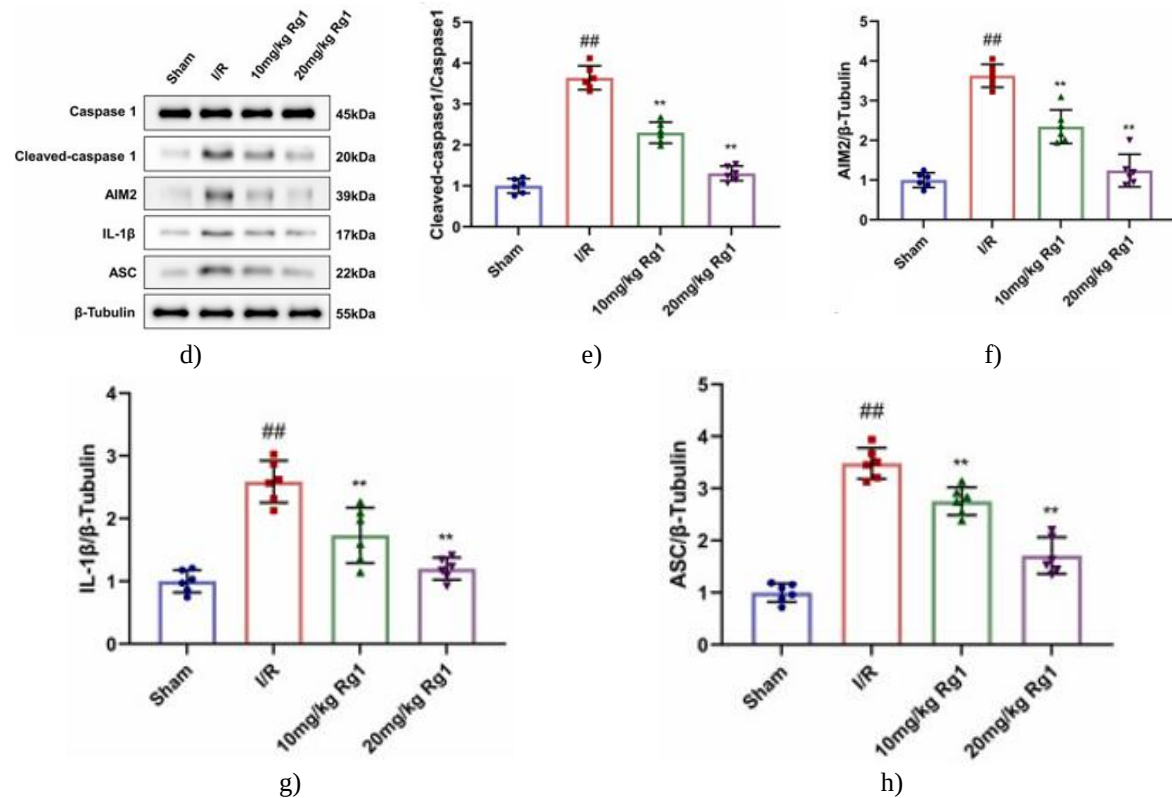


Figure 2. Modulatory action of Rg1 on cardiac inflammation triggered by MI/R injury. (a–c) Serum transcription profiles of IL-1β, TNF-α, and IL-10 mRNA; (d) Immunoblots derived from rat myocardial tissue lysates; (e–h) Relative protein abundance of Cleaved-caspase1, AIM2, IL-1β, and ASC. Data are presented as means ± SD; ##P < 0.01 vs. Sham group, *P < 0.05, **P < 0.01 vs. I/R group.

Immunoblotting confirmed that the protein translation of Cleaved-caspase-1, AIM2, IL-1β, and ASC escalated drastically within the myocardial tissues of the MI/R model rats, whereas the introduction of Rg1 effectively forced a downward trend in their expression levels (**Figures 2d and 2h**). Taken together, these data imply that pre-treatment with Rg1 serves to blunt the acute inflammatory response driven by MI/R stress.

Rg1 suppresses macrophage M1 polarization during MI/R injury

The enzymes iNOS and Arg-1 function as representative phenotypic markers for M1 pro-inflammatory and M2 anti-inflammatory macrophage activation states, respectively. Mechanistic exploration demonstrated that iNOS expression rose substantially under MI/R conditions; however, this trend was prominently reversed, while Arg-1 expression was concurrently enhanced, in the cohorts receiving Rg1 compared to the unmanaged MI/R model animals (**Figures 3a and 3c**).

Debris such as naked DNA released from necrotic cardiomyocytes is known to provoke the activation of the AIM2 inflammasome, which subsequently steers macrophages toward an M1 phenotype. To visualize this in situ, immunohistochemical analysis was performed on rat cardiac sections; the cell nuclei and surrounding myofibrils in the sham group retained an ordered, linear configuration, with the AIM2 protein uniformly scattered throughout the tissue. In contrast, the MI/R cohort exhibited hyperactivation of the AIM2 macromolecular complex, alongside a profound escalation in the spatial co-localization of the macrophage marker F4/80 with AIM2. This pathological phenotype was distinctly suppressed in the Rg1 intervention group (**Figures 3d and 3g**). These observations collectively signal that Rg1 exposure hampers both pro-inflammatory M1 macrophage shifting and the induction of the AIM2 complex.

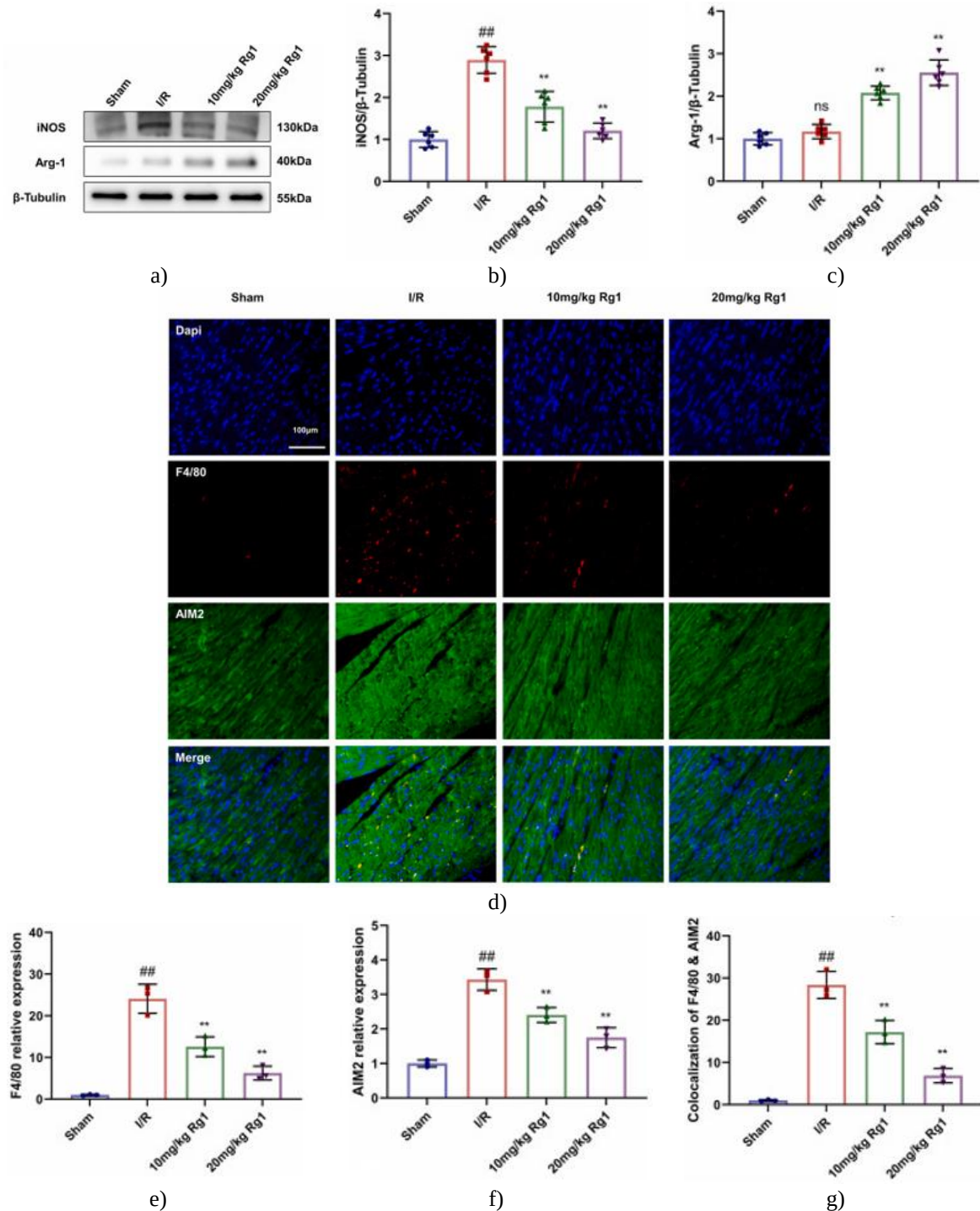


Figure 3. Rg1 limits macrophage M1 phenotypic shifting during MI/R injury. (a) Immunoblot profiles of cardiac tissue specimens; (b–c) Quantitative expression metrics for iNOS and Arg-1; (d) Immunohistochemical evaluation of myocardial sections (scale bar = 100 μ m); (e–f) Relative expression values of the macrophage marker F4/80 and the inflammasome component AIM2 in heart samples; (g) Immunofluorescence micrographs documenting the co-localization dynamics of F4/80 and AIM2. Data are presented as means $\pm$ SD; ##P < 0.01 vs. Sham group, **P < 0.01 vs. I/R group.

Rg1 limits myocardial fibrosis following MI/R injury

The translation levels of key fibrotic structural markers—namely FN, Collagen α -1, and α -SMA—were monitored in cardiac tissue lysates using Western blotting. The abundance of these proteins grew exponentially in the unmanaged MI/R group, but was significantly driven down upon Rg1 administration (**Figures 4a and 4d**).

To track these structural alterations, Masson's trichrome staining was performed on the myocardial tissue. Under this stain, cellular nuclei and collagen networks show a blue coloration, whereas the cytoplasmic matrix, muscle bundles, and erythrocytes appear red. The cardiac architecture of the MI/R group revealed an extensive accumulation of blue collagenous fibers. Conversely, the rats under Rg1 treatment exhibited a marked reduction in these deposits alongside preserved cardiomyocyte integrity, demonstrating that Rg1 acts as a powerful inhibitor of the tissue remodeling and fibrosis triggered by MI/R injury (**Figures 4e and 4f**).

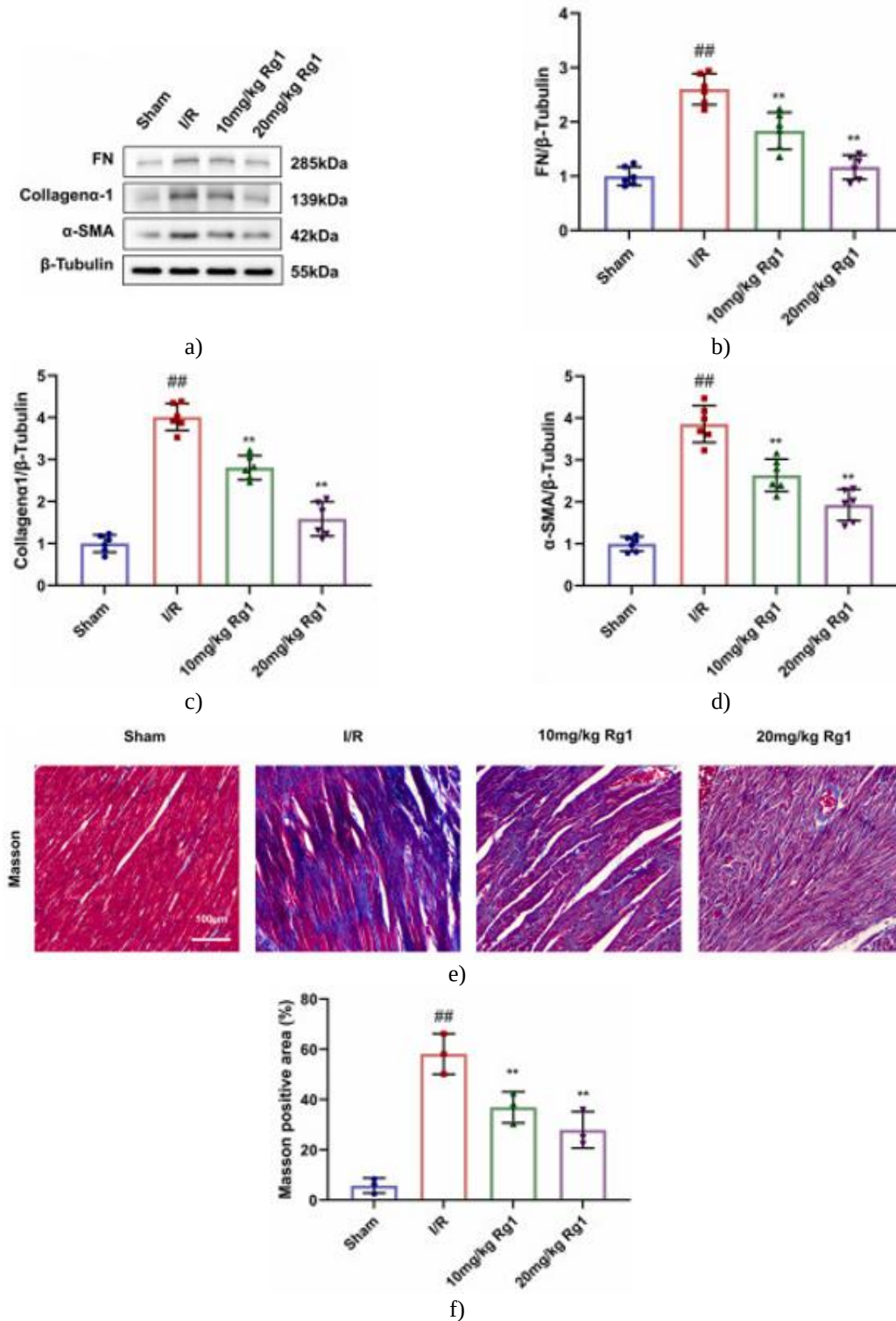


Figure 4. Influence of Rg1 on the progression of cardiac fibrosis under MI/R conditions. (a) Immunoblotting from myocardial tissue samples; (b–d) Densitometric protein expression calculations for FN, Collagenα-1,

and α -SMA; (e) Masson's trichrome histochemical tracking of fibrotic changes (scale bar = 100 μ m); (f) Percentage of Masson-positive fibrotic surface area. Data are presented as means $\pm$ SD; ###P < 0.01 vs. Sham group, **P < 0.01 vs. I/R group.

Rg1 blocks AIM2 inflammasome assembly in vitro

To establish whether the regulatory control of ginsenoside Rg1 over the AIM2 inflammasome persists in an isolated environment, we tracked the formation of ASC specks (a standard indicator of active inflammasome assembly) along with the expression of the upstream components AIM2 and IL-1 β . Immunofluorescence microscopy confirmed that the generation of macromolecular ASC specks within bone marrow-derived macrophages (BMDMs) expanded dramatically following exposure to exogenous necrotic cell DNA. Crucially, the addition of Rg1 caused a sharp, significant decline in speck frequency compared to the challenged model group (**Figures 5a and 5b**).

Western blot evaluations reinforced these findings; the protein levels of Cleaved-caspase 1, AIM2, IL-1 β , and ASC in the necrotic DNA-exposed model cells were vastly superior to those found in the baseline control cells. Nevertheless, treating the cells with Rg1 successfully down-regulated the production of this entire protein panel, validating that Rg1 directly arrests the operational activation of the AIM2 inflammasome in vitro (**Figures 5c and 5g**).

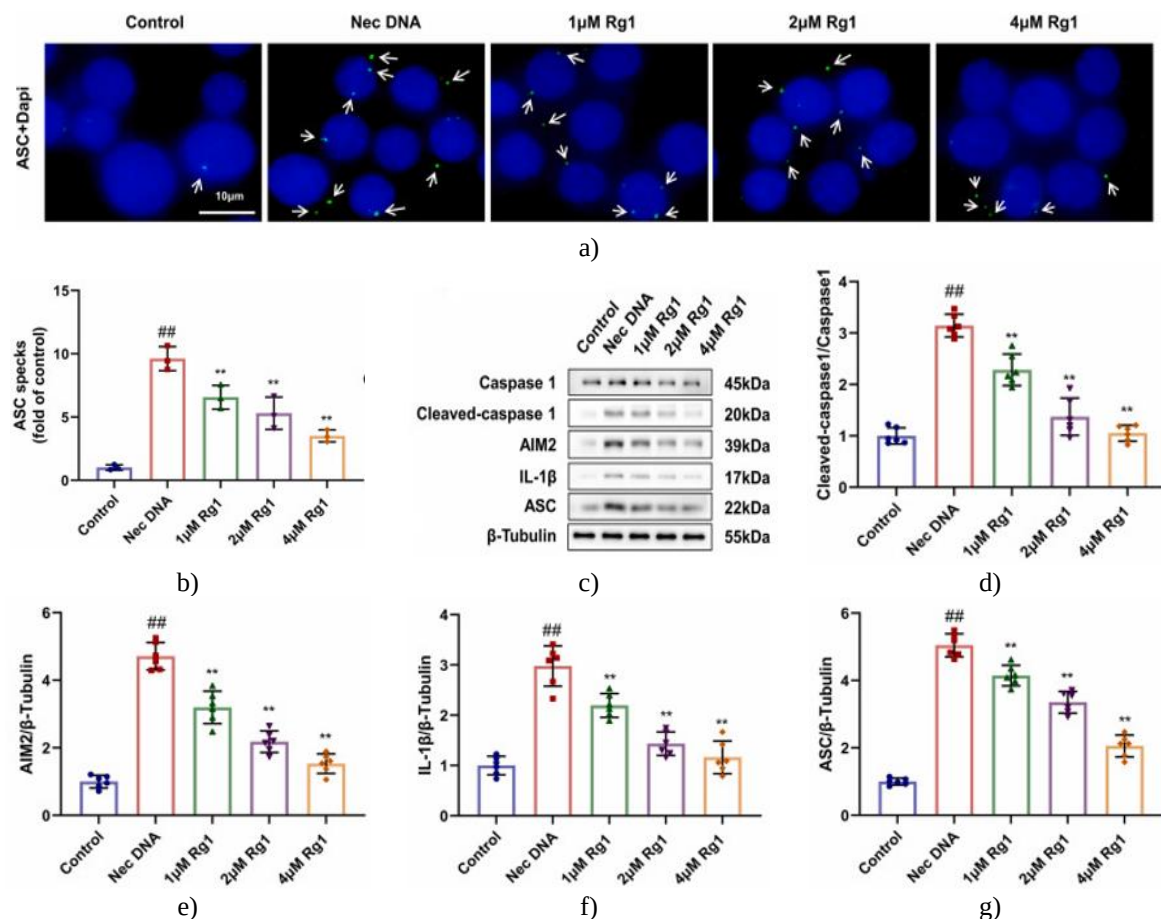
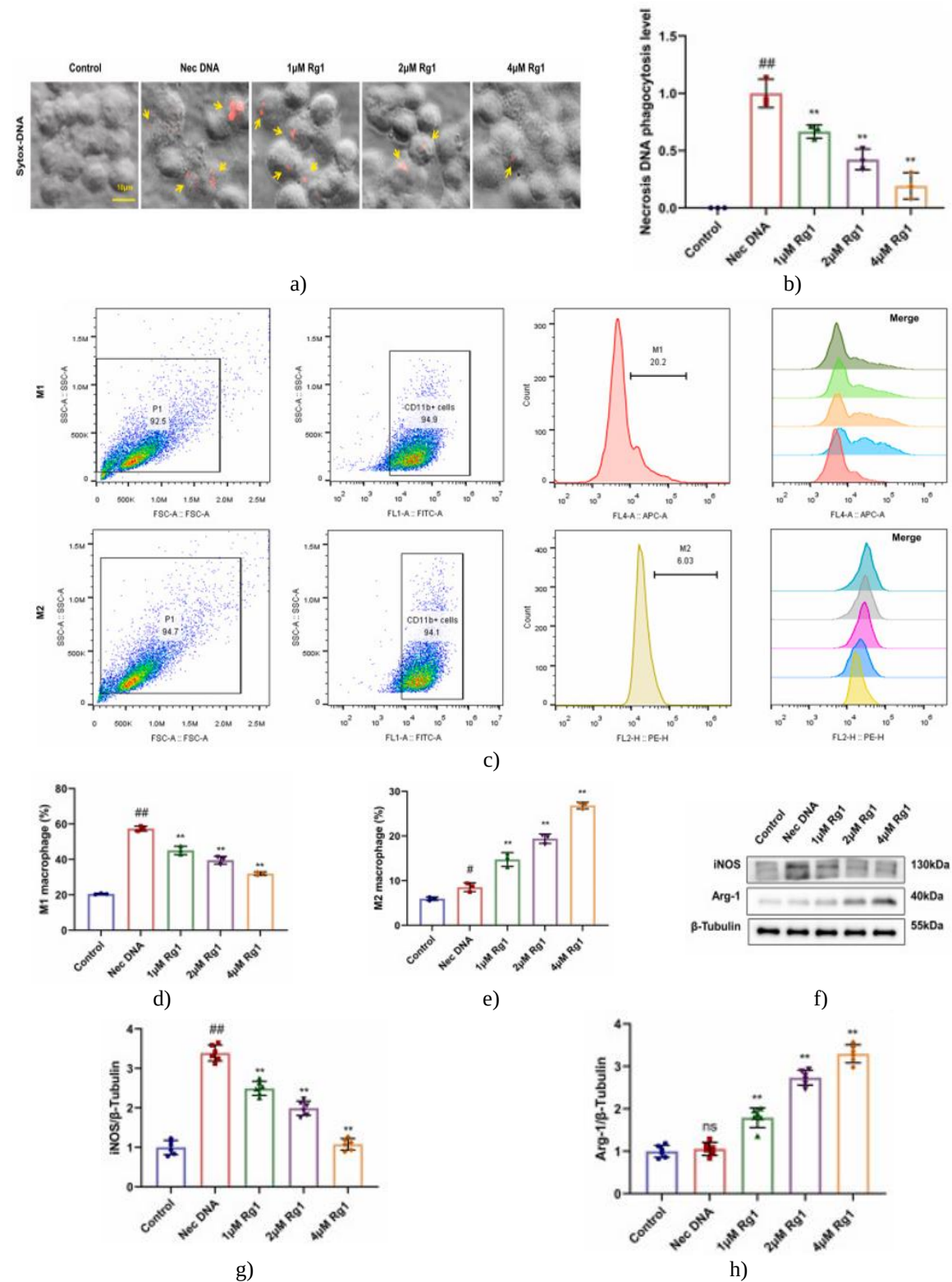


Figure 5. Suppressive profile of Rg1 against AIM2 inflammasome induction in vitro. (a) Immunocytochemical visualization showing the outcomes of different Rg1 concentrations on stimulated BMDMs (scale bar = 10 μ m); (b) Statistical tracking of ASC speck numbers (expressed relative to control values); (c–g) Densitometric values for Cleaved-caspase 1, AIM2, IL-1 β , and ASC proteins. Data are presented as means $\pm$ SD; ###P < 0.01 vs. Control group, **P < 0.01 vs. Nec DNA group.

Rg1 suppresses in vitro macrophage M1 polarization

To evaluate macrophage functionality, BMDMs were incubated with SYTOX Orange nucleic acid stain, which selectively labels the DNA of necrotic macrophages with red fluorescence. No positive staining was detected

within the baseline control cohort. Conversely, the experimental group exposed to necrotic cell DNA demonstrated a pronounced elevation in both macrophage-specific markers and internalized DNA particles, whereas concurrent administration of Rg1 precipitated a concentration-dependent decline in these parameters (**Figures 6a and 6b**). These observations reveal that Rg1 intervention down-regulates the capacity of macrophages to undergo DNA phagocytosis.



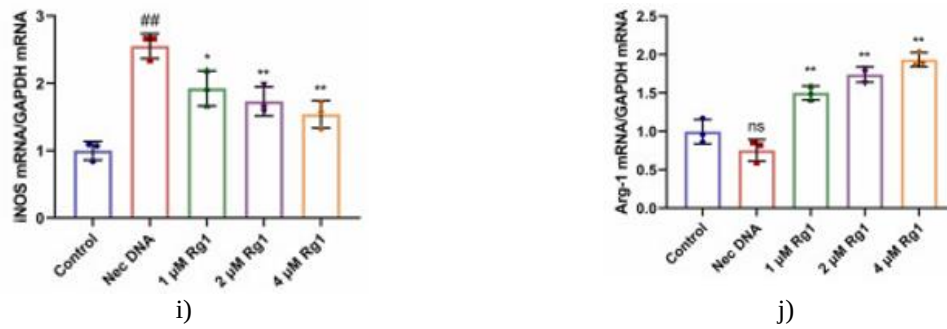
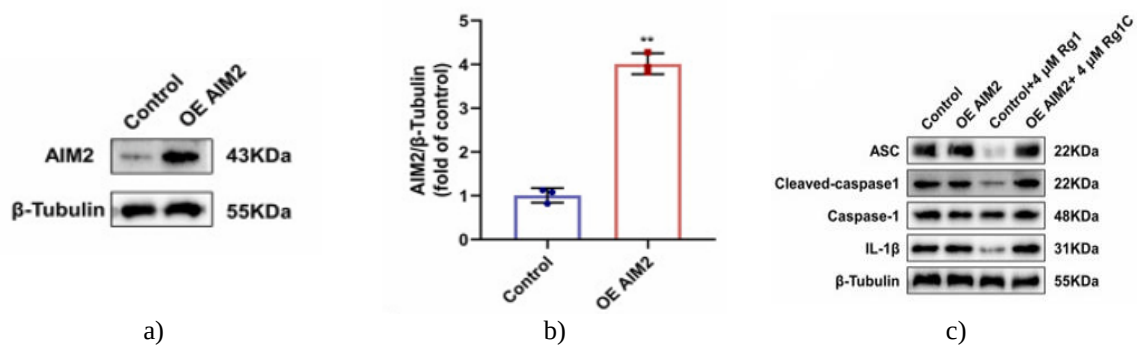


Figure 6. Suppressive action of Rg1 on the in vitro M1 phenotypic shifting of macrophages under simulated MI/R conditions. (a) SYTOX Orange staining profiles of murine BMDMs (scale bar = 10 μm); (b) Quantification of DNA engulfment by macrophages; (c–e) Flow cytometric assessment of macrophage M1/M2 surface markers and their relative cellular frequencies; (f) Representative immunoblots; (g–h) Densitometric protein expression levels of iNOS and Arg-1; (i–j) Relative mRNA transcript abundance of iNOS and Arg-1. Data are expressed as means ± SD; ##P < 0.01 compared to the Control group, **P < 0.01 compared to the Nec DNA group.

Immunofluorescence imaging and flow cytometric profiling revealed no statistically significant deviations between the baseline control group and the model cohort challenged solely with necrotic cell DNA. However, treatment with Rg1 initiated a dose-dependent reduction in the fraction of M1-polarized macrophages, which was mirrored by a concomitant expansion in the M2-polarized macrophage population (**Figures 6c and 6e**). At the translational level, iNOS protein abundance surged markedly in the model group relative to controls; yet, this pro-inflammatory induction was significantly suppressed following Rg1 intervention, which simultaneously promoted a robust upregulation of Arg-1 protein expression (**Figures 6f and 6h**). These proteomic shifts were fully corroborated by qRT-PCR transcript analyses (**Figures 6i and 6j**). Collectively, these in vitro findings replicate the prior in vivo observations, substantiating that Rg1 treatment effectively dampens M1 macrophage polarization.

Rg1 hampers macrophage M1 polarization via suppression of the AIM2 inflammasome pathway

To definitively substantiate the underlying molecular pathway of Rg1, an AIM2-overexpressing plasmid construct was utilized. Effective cellular transfection was validated via Western blot analysis (**Figures 7a and 7b**). Upon up-regulation of AIM2, the expression levels of ASC, cleaved-caspase 1, and total caspase-1 were noticeably augmented in the OE AIM2 + 4 μM Rg1 cohort when compared against the Control + 4 μM Rg1 group, establishing that overexpressing AIM2 counteracts the suppressive influence of Rg1 on inflammasome assembly. Concurrently, tracking the classic macrophage polarization determinants revealed an elevation in iNOS expression alongside a marked reduction in Arg-1 levels. These findings illustrate that driving up AIM2 inflammasome activity subsequently accelerates pro-inflammatory M1 macrophage shifting, thereby overriding the protective, suppressive effects of Rg1. In summary, these data confirm that Rg1 hinders the M1 polarization of macrophages by directly blocking the functional activation of the AIM2-ASC-caspase-1 inflammasome axis.



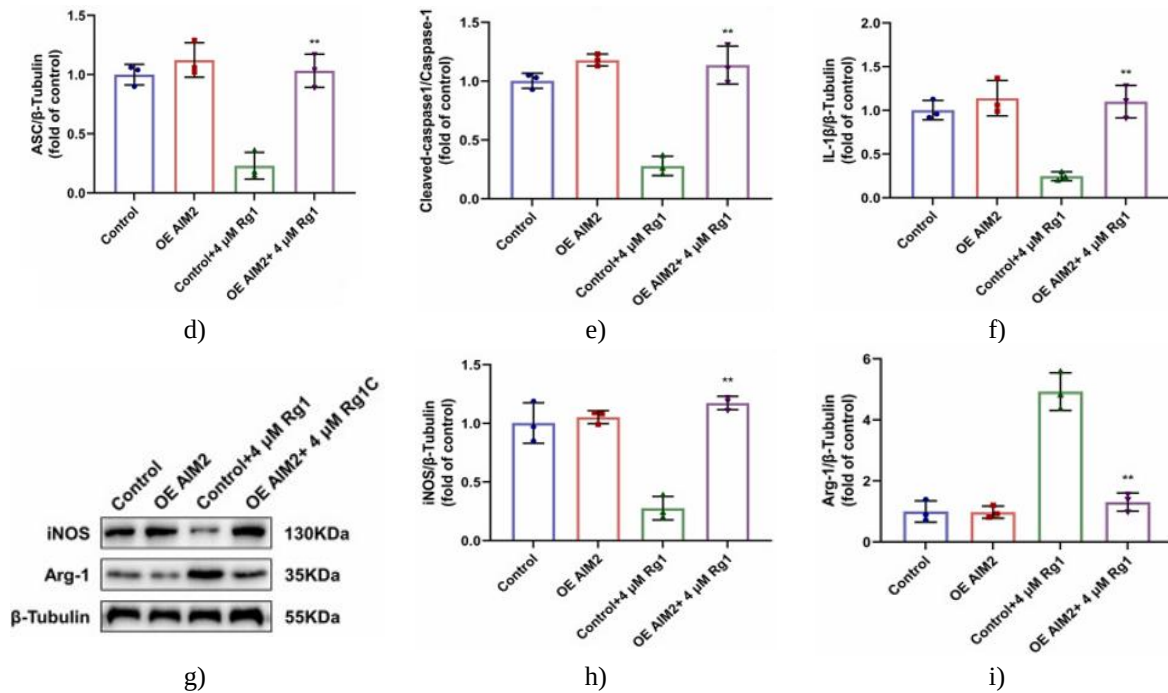


Figure 7. Abrogation of the protective properties of Rg1 under conditions of in vitro AIM2 overexpression. (a–b) Intracellular abundance of AIM2 within engineered murine BMDMs; (c) Immunoblotting analysis from treated BMDM lysates; (d–f) Densitometric quantification of ASC, cleaved-caspase 1, and IL-1β protein expressions; (g) Western blot analysis of macrophage lysates; (h–i) Proteomic expression patterns of iNOS and Arg-1. Data are expressed as means ± SD; **P < 0.01 compared to the OE AIM2 group.

In this study, we demonstrated that Rg1 mitigates myocardial inflammation and fibrosis resulting from myocardial I/R injury via the suppression of macrophage polarization. The inflammatory cascade represents a vital component in the initiation and progression of tissue damage. Specifically, MI/R triggers both the secretion of diverse pro-inflammatory mediators and the infiltration of inflammatory cells, culminating in myocardial dysfunction and tissue necrosis [24]. This response is a major driver of adverse left ventricular remodeling in individuals suffering from myocardial infarction. Consequently, suppressing the inflammatory response represents an essential strategy for diminishing MI/R injury and enhancing clinical outcomes for patients with myocardial infarction. Our results emphasize the diverse cardioprotective properties of Rg1, highlighting its prospective utility as a therapeutic agent to alleviate the adverse outcomes of MI/R injury by suppressing inflammation, modulating macrophages, and minimizing fibrosis.

Because of their profound consequences on global public health, MI/R injuries continue to be a primary focus of scientific inquiry. The contribution of inflammasomes to MI/R injury has increasingly become a subject of scientific interest. Inflammasomes function as multiprotein complexes that react to cellular stress by activating caspase-1, which subsequently prompts the secretion of the pro-inflammatory cytokines IL-1β and IL-18. Multiple distinct inflammasomes, such as NLRP3 and AIM2, are involved in intensifying myocardial damage during the reperfusion phase [25]. Acting as an primary detector of danger signals following myocardial ischemic injury, the AIM2 inflammasome undergoes widespread activation after myocardial ischemic infarction and helps govern post-injury myocardial fibrosis [26, 27]. Consequently, targeting these specific inflammasomes offers a promising strategy for dampening inflammatory processes and mitigating myocardial damage.

Macrophages serve as central components during the MI/R process, and their specific polarization phenotype substantially dictates cardiac outcomes. M2 macrophages possess anti-inflammatory characteristics and suppress myocardial inflammation through the secretion of IL-10 [28]. The equilibrium maintained between M1 and M2 macrophage populations impacts the extent of tissue destruction and subsequent healing during MI/R. Interventions designed to modulate macrophage polarization could therefore offer therapeutic utility by encouraging tissue restoration and restricting inflammation-driven damage. This intricate relationship among inflammasomes, macrophage polarization, and cardiac damage has been progressively clarified by recent investigations into MI/R [29]. Furthermore, numerous pattern recognition receptors expressed by macrophages

trigger inflammatory pathways [5], and these cells also fulfill a primary role in organizing inflammatory cascades [30, 31]. Deciphering these complex molecular mechanisms introduces novel possibilities for targeted therapeutic strategies that could fundamentally transform the clinical management of MI/R injuries and enhance patient recovery. Nonetheless, additional investigations are required to elucidate the exact signaling cascades involved and to confirm the clinical efficacy and safety of these proposed interventions.

Ginsenoside Rg1, which is well-documented for its defensive capabilities against cerebral ischemic damage, has attracted substantial interest within the field of cardiovascular medicine [32-34]. Ginsenosides deliver their cardioprotective benefits through multiple distinct pathways, and our research offers deeper comprehension regarding the specific functional mechanics of Rg1. Notably, ginsenosides have demonstrated encouraging outcomes in obstructing the activation of inflammasomes like AIM2, which leads to a decrease in the secretion of pro-inflammatory cytokines. As documented in a prior investigation [35], ginsenosides Rg1 and Rh3—the primary active components of RGE—markedly suppress macrophage pyroptosis by blocking AIM2 inflammasome activation. This anti-inflammatory capability holds considerable promise for dampening the inflammatory cascades triggered by myocardial ischemia and the resulting tissue damage, thereby facilitating myocardial recovery.

The present study offers further confirmation that an intrinsic mechanism underpins the protective capabilities of Rg1 against MI/R damage. In contrast to the protective pathways reported for other pharmaceuticals in recent literature [36-38], this investigation verifies that Rg1 can mitigate MI/R injury by preventing the activation of the AIM2 inflammasome, which consequently restrains macrophage polarization. This insight offers valuable strategies for the cardiovascular field as research transitions from animal models to clinical therapies. Additionally, this work serves as a foundational benchmark for investigating how ginsenosides function in the management of inflammatory tissue damage [23]. Nevertheless, because this research relied on *in vitro* cellular systems and animal models, the feasibility of utilizing Rg1 as a monotherapy in clinical practice remains unverified. Furthermore, while Rg1 displayed a robust capacity to block AIM2 inflammasome activation, its selective inhibitory properties regarding the activation of alternative inflammasomes, such as NLRP3, require further clarification.

Conclusion

To conclude, our investigation establishes that Rg1 exerts dose-dependent cardioprotective actions against MI/R injury by dampening inflammation, preventing AIM2 inflammasome activation, and blocking M1 macrophage polarization while safeguarding cardiac structural integrity. These outcomes point to its viability as a prospective therapeutic candidate for treating MI/R injury.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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