

Research Article

GA Eases Atherosclerosis by Regulating Circadian Rhythm

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Abstract

Background: Atherosclerosis (AS) serves as a fundamental high-risk factor associated with the development of cardiovascular diseases. There is an urgent need to investigate efficacious therapeutic strategies and molecular targets to combat AS. **Methods:** Apolipoprotein E (ApoE)^{−/−} mice fed a high-fat diet were administered glycyrrhetic acid (GA) to establish the AS intervention model. An enzyme-linked immunosorbent assay (ELISA) was used to detect serum levels of the pro-inflammatory cytokines IL-6 and tumor necrosis factor- α (TNF- α). Concurrently, biochemical analyses measured serum lipid profiles, including low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and total cholesterol (TC). Total RNA extracted from mouse aortic tissues was subjected to RNA sequencing to identify differentially expressed genes (DEGs), followed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. Cytoscape software, combined with weighted gene co-expression network analysis (WGCNA), was used to filter candidate target genes, and quantitative real-time PCR (qPCR) was conducted to verify the expression levels of hub clock genes. **Results:** GA markedly suppressed the overproduction of IL-6 and TNF- α in AS model mice. Meanwhile, GA reduced serum LDL-C, TG, and TC concentrations and elevated HDL-C levels. RNA-seq-derived DEGs were significantly enriched in biological processes and signaling pathways related to circadian rhythm. Three core clock genes, period circadian clock 1 (*Per1*), period circadian clock 2 (*Per2*), and period circadian clock 3 (*Per3*), were identified as candidate targets. qPCR results validated that GA significantly upregulated the expression of *Per1*, *Per2*, and *Per3* in aortic tissues. **Conclusion:** This study preliminarily confirms that GA exerts anti-atherosclerotic effects in ApoE^{−/−} mice. Its potential therapeutic mechanism may be associated with circadian rhythm-related signalling involving *Per1*, *Per2*, *Per3*.

Keywords: atherosclerosis; glycyrrhetic acid; circadian rhythms; RNA sequence analysis

1. Introduction

The prevalence of atherosclerosis increases significantly with advancing age. Key risk factors include hypertension, hyperlipidemia (especially elevated low-density lipoprotein cholesterol (LDL-C)), diabetes mellitus, among others. Recent epidemiological trends indicate a shift toward younger populations affected by the condition [1]. The pathogenesis of atherosclerosis is characterized by a chronic multifactorial process, involving endothelial damage, abnormal lipid accumulation, and persistent inflammatory responses acting in concert [2]. Lipid-lowering agents (such as statins) are among the most commonly used medications for the prevention and treatment of atherosclerosis; however, they are not without potential adverse effects, including myopathy and disturbances in glucose metabolism [3]. Advancements in research have highlighted the potential therapeutic roles of certain traditional Chinese medicines such as *Salvia miltiorrhiza* and *Panax notoginseng*—primarily through anti-inflammatory, antioxidant, and lipid-modulating mechanisms [4,5]. Nonetheless, despite

these promising findings, the precise molecular mechanisms underlying the efficacy of these traditional therapies warrant further investigation.

Since ancient times, licorice has been widely used as a medicinal herb. Its root exhibits a broad spectrum of pharmacological properties, including adrenocortical hormone-like actions, and effects on the digestive, immune, and cardiovascular systems [6]. Glycyrrhetic acid (GA), an oleanane-type pentacyclic triterpene, is the principal bioactive constituent of licorice. This compound exhibits a diverse pharmacological profile, encompassing anticancer, antioxidant, anti-inflammatory, and hepatoprotective effects [7,8,9]. In the context of cardiovascular diseases, cumulative evidence indicates that GA mitigates Dox-induced cardiotoxicity by activating the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) signaling pathway. This mechanism potentially suppresses oxidative stress, mitochondrial dysfunction, and apoptotic cascades [10]. Furthermore, GA has been shown to inhibit tumor necrosis factor- α (TNF- α)-stimulated expression of intercellular adhesion molecule-1 (ICAM-1) in human um-



bilical vein endothelial cells (HUVECs) [11]. Collectively, these findings underscore the therapeutic potential of GA in managing inflammation-associated cardiovascular disorders.

The circadian rhythm is an intrinsic timekeeping system, maintained by a molecular feedback loop involving core clock genes [12]. These genes integrate with environmental cues to orchestrate rhythmic metabolic, immune, and other physiological processes [13]. Emerging evidence highlights the critical role of circadian regulation in the pathogenesis of atherosclerosis, across multiple dimensions: glucose and lipid homeostasis, endothelial dysfunction, vascular smooth muscle cell (VSMC) phenotypic transition, and inflammatory and immune responses [14,15,16]. However, the precise mechanisms through which specific clock genes modulate atherosclerotic progression remain incompletely understood.

In this study, serum biochemical profiling was conducted on atherosclerotic model mice and GA-treated counterparts. Transcriptomic analyses were further conducted to identify hub differentially expressed genes (DEGs) and characterize their expression dynamics within the atherosclerotic lesions. The study aimed to elucidate the molecular mechanisms of GA in atherosclerotic mice and identify novel pathways through which GA modulates circadian rhythms to impede atherosclerotic progression.

2. Materials and Methods

2.1 Chemicals and Reagents

18 β -glycyrrhetic acid (GA; purity $\geq 97\%$; catalog no. G10105) was purchased from Merck (Darmstadt, Germany). All enzyme-linked immunosorbent assay (ELISA) kits were obtained from Shanghai Enzyme-Linked Biotechnology Co., Ltd. (Shanghai, China).

2.2 Animal Experiment

Shanghai Bikai Keyi Biotechnology Co., Ltd. (Shanghai, China) supplied male C57BL/6 and ApoE^{-/-} mice (4 C57BL/6 mice and 16 ApoE^{-/-} mice). Male C57BL/6 mice were used as the control group, and male ApoE^{-/-} mice were allocated to the atherosclerosis model group and the GA treatment group. All mice were 8 weeks old with body weights ranging from 20 to 22 g at the start of the experiment. All animals were housed under standard specific pathogen-free (SPF) conditions, and body weight fluctuations were monitored throughout the experimental period. This animal experiment received ethical clearance from the Animal Ethics Committee of Changchun University of Chinese Medicine (No. 2025294). Mice were reared in Specific-pathogen-free (SPF) animal rooms at 23 $\pm$ 2 °C with 44%–52% humidity and a 12 h light-dark cycle. ApoE^{-/-} mice were fed a high-fat diet (HFD, consisting of 60% kcal fat, 20% kcal carbohydrate, and 20% kcal protein, Product No. D12492, Research Diets, Inc., New Brunswick, NJ, USA) for 12 weeks to in-

duce atherosclerosis (AS). After one-week acclimatisation, numbered mice were randomly assigned to five groups using the built-in random-number generator in GraphPad Prism 9.0. No a-priori inclusion/exclusion criteria were predefined; animals with severe weight loss, spontaneous disease or trauma would have been excluded, but none were encountered in this study. For outcome-assessment blinding, samples were labelled with anonymous numeric IDs. Operators for serum biochemistry, ELISA and qPCR were blinded to group allocation, whereas staff performing grouping, gavage and tissue collection knew group assignments. Bioinformatic analysts processed RNA-seq raw data using coded identifiers; group information was revealed after primary data processing was finished. The mice were divided into 5 groups: the Control group (C57BL/6 mice fed a normal chow diet), the AS group, and the GA-5, 10, 20 mg/kg groups (ApoE^{-/-} mice were induced with AS, followed by 5, 10, 20 mg/kg GA treatment for 8 weeks). GA was prepared by dissolving GA powder in distilled water. Mice were intragastrically administered with GA at gradient doses of 5, 10, and 20 mg/kg, with a uniform administration volume of 10 mL/kg, once daily. All experimental mice were fasted for 12 h with free access to drinking water after the final GA administration. Blood and aortic tissues were collected at a fixed time point on the following day to avoid experimental bias caused by circadian rhythm.

All mice were anesthetized via intraperitoneal injection of pentobarbital sodium solution (50 mg/mL) at a dose of 50 mg/kg. Loss of corneal and paw withdrawal reflexes was verified to confirm full anesthesia before blood and aortic tissue collection. Following sampling, mice were euthanized with an intraperitoneal overdose of pentobarbital sodium at 200 mg/kg (50 mg/mL stock solution). Permanent death was confirmed by the complete absence of spontaneous respiration and heartbeat for at least 5 min. Then, blood and aorta were collected and stored for analysis.

2.3 Serum Biochemical Analysis

The collected blood was subsequently centrifuged at 2000 $\times$ g for 15 min to extract the serum. The serum was diluted with deionized water at a 4:1 ratio. An iMagic-V7 biochemical analyzer (Kubel Biotechnology, Shenzhen, China) was used to measure serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), LDL-C, and triglycerides (TG) (n = 4 per group).

2.4 Enzyme-Linked Immunosorbent Assay

Serum concentrations of TNF- α and IL-6 were measured using commercially available ELISA kits (Boyan Biotech, Shandong, China) following the manufacturer's instructions (n = 4 per group).

2.5 RNA Sequencing

Total RNA was extracted from murine aorta (n = 4 per group) using TRIzol reagent, followed by reverse tran-

Table 1. Specific primers used for quantitative real-time PCR.

Gene	Forward (5'–3')	Reverse (5'–3')
<i>Per1</i>	AGGTGGCTTTCGTGTTGG	CAATCGATGGATCTGCTCTGAG
<i>Per2</i>	TGGACACAGAGAACGAGATGG	CAGGAAGCGGTAGGTGAAGA
<i>Per3</i>	CTGGACATCCTGGACCTGTT	GGCTTCTTCTGGATGTCGTC
<i>β-actin</i>	TGGCACCCAGCACAATGAA	CTAAGTCATAGTCCGCCTAGAAGCA

Per1, Period circadian clock 1; *Per2*, Period circadian clock 2; *Per3*, Period circadian clock 3.

scription and amplification in accordance with established protocols. Sequencing libraries were constructed on the BGISEQ-500 platform (Novogene, Beijing, China). RNA quality was evaluated using an Agilent Bioanalyzer 2100 (Agilent, Santa Clara, California, USA), evaluating parameters such as mRNA concentration, RNA Integrity Number (RIN), and 28S/18S rRNA ratio. Post-quality control, RNA was reverse-transcribed into complementary DNA (cDNA) using the SMART-Seq HT kit (Takara Bio, San Jose, California, USA), and then library construction and sequencing were carried out.

Raw sequencing data were filtered with Short Oligonucleotide Analysis Package nuke (SOAPnuke) to remove adapter-containing reads, and the resulting clean reads were stored in FASTQ format. Transcript reads were aligned to the mouse reference genome (GRCm38) using Hierarchical Indexing for Spliced Alignment of Transcripts 2 (HISAT2) and Spliced Transcripts Alignment to a Reference (STAR) RNA-seq aligners, and transcripts mapped to genes were further quantified. Normalized mRNA expression levels for each sample were calculated accordingly. To identify DEGs, DESeq2 was used to analyze the expression profiles of all mapped genes. Genes were identified as differentially expressed using thresholds of false discovery rate (FDR) <0.05 and p -value < 0.05. All RNA-seq-related procedures, including RNA extraction, quantification, and cDNA library preparation, were performed by Novogene using standardized and consistent protocols.

2.6 Enrichment Analysis

The genes in the 3 groups were compared in pairs, including the AS group vs. the Control group and the GA-20 mg/kg group vs. the AS group. Genes with expression levels that met the criteria of $|\log_2(\text{FoldChange})| > 1$ and $p < 0.05$ were considered to be DEGs. The NovoMagic website (<https://magic-plus.novogene.com>; Novogene, Beijing, China) was used for Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analyses of DEGs.

2.7 Target Gene Screening

DEGs were imported into the STRING database (<http://cn.string-db.org/>) to generate protein-protein interaction (PPI) networks. The resulting PPI networks were then imported into Cytoscape, and the cytoHubba plugin was used to identify the top 50 genes. These 50 genes were further an-

alyzed with the MCODE algorithm to identify key modules and extract Hub genes. To enhance the accuracy of the results, DEGs were subjected to weighted gene co-expression network analysis (WGCNA, <https://magic-plus.novogene.com>) to identify corresponding core genes.

Finally, the Hub genes identified via Cytoscape and the core genes derived from WGCNA were intersected to determine the target genes.

2.8 Quantitative Real-Time PCR

Aortic tissue homogenates were prepared for total RNA isolation utilizing the RNA Easy Fast Animal Tissue/Cell Total RNA Extraction Kit (Catalog No. DP451, Tiangen Biotech, Beijing, China). RNA concentration was quantified with a NanoDrop One C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Purified RNA was subsequently reverse-transcribed into cDNA with FastKing gDNA Dispelling RT Supermix (Catalog No. KR118, Tiangen Biotech). SYBR Green-based quantitative real-time PCR (qPCR) was carried out on a BioRad-CFX96 thermal cycler with SuperReal Premix Plus (Catalog No. FP205, Tiangen Biotech). Primer sequences for the target and reference genes (β -Actin) are listed in Table 1. The $2^{-\Delta\Delta C_t}$ algorithm was applied to compute relative mRNA expression levels ($n = 4$ per group).

2.9 Statistical Analysis

All experimental data were presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software, LLC, Boston, Massachusetts USA), and intergroup comparisons across multiple cohorts were conducted using one-way analysis of variance (ANOVA). Statistical significance was defined as $p < 0.05$.

3. Results

3.1 GA Regulates Transcription in the Aorta of AS Mice

Mice were subjected to a HFD to induce AS, then treated with GA for 8 weeks, followed by aortic RNA sequencing. As shown in Fig. 1A, the gene expression profiles for each sample group were presented via a box plot. As shown in Fig. 1B,C volcano plots, there were 1076 DEGs between the AS and Ctrl groups, whereas 3297 DEGs were identified between the GA and AS groups, with 1592 genes upregulated and 1705 genes downregulated. Subsequently, the intersection of these two DEG sets was deter-

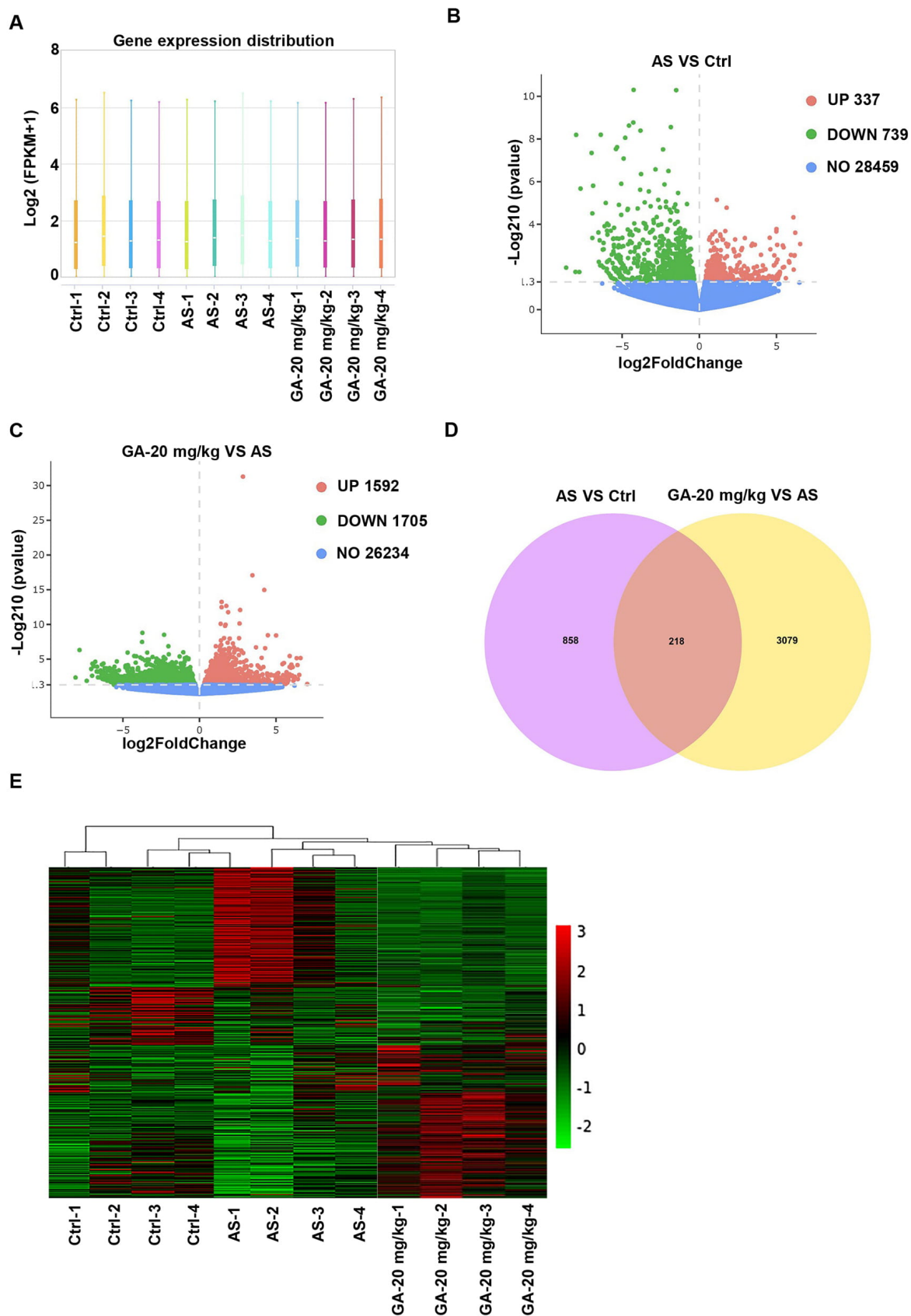


Fig. 1. RNA sequencing analysis of the aorta of AS mice treated with GA. (A) Box plots showed gene expression distribution. (B,C) DEGs are depicted in a volcano plot. (D) Co-DEGs are shown in a Venn diagram. (E) Co-DEGs clustering heat map. AS, atherosclerosis; GA, glycyrrhetinic acid; DEGs, differentially expressed genes.

mined, resulting in 218 common DEGs, as shown in Fig. 1D. These co-DEGs were further analyzed through hierarchical clustering and visualized using a heat map in Fig. 1E. The findings demonstrate significant alterations in gene expression within the aorta of atherosclerotic mice.

3.2 GA Regulates the Circadian Rhythm of AS Mice

To explore the mechanisms through which GA plays a role, we performed GO and KEGG enrichment analyses on co-DEGs. The results of the GO analysis were shown in Fig. 2A. Co-DEGs were found to be involved in biological processes such as rhythmic processes, photoperiod-driven entrainment of the circadian clock by photoperiod, and circadian regulation of gene expression. As shown in Fig. 2B, the co-DEGs involved in major biological processes were displayed in a chord diagram. The co-DEGs involved in rhythm-related biological processes included *Per1*, *Per2*, *Per3*, and ubiquitin specific peptidase 2 (*Usp2*). KEGG enrichment analysis revealed that co-DEGs were enriched in multiple rhythm-related pathways including Circadian rhythm and Circadian entrainment (Fig. 2C). As shown in the chord diagram (Fig. 2D), the main co-DEGs involved in rhythm-related pathways were D site albumin promoter binding protein (*Dbp*), *Per1*, *Per2*, *Per3*, neuronal PAS domain protein 2 (*Npas2*), Fos proto-oncogene (*Fos*), and guanylate cyclase 1, soluble, beta 2 (*Gucy1b2*). The above results suggested that the therapeutic effect of GA on AS may be related to its regulation of circadian rhythmic processes.

3.3 Identification of Potential Target Genes of GA

To identify potential GA target genes, we screened for co-DEGs using two methods. Initially, co-DEGs were uploaded to the STRING database for PPI network analysis (Fig. 3A). Subsequently, the PPI network was analyzed using the CytoHubba module in Cytoscape. These top 50 genes were then identified as hub genes (Fig. 3B). These 50 genes were subjected to key module extraction using MCODE, yielding the following modules: *Per1*, *Per2*, *Per3*, WEE1 homolog 1 (*Wee1*), *Usp2*, *Dbp*, hypoxia-inducible factor (*Hif*), *Npas2*, and *Ciart* (Fig. 3C). As shown in Fig. 3D, we further analyzed the co-DEGs using WGCNA and screened out 45 core genes. These 45 genes were then subjected to key module extraction, obtaining 10 genes: *Per1*, *Per2*, *Per3*, microfibrillar associated protein 5 (*Mfap5*), X-inactive specific transcript (*Xist*), gastrin 3 (*Gkn3*), fibronectin type III domain containing 1 (*Fndc1*), LRRN4 C-terminal like (*Lrrn4cl*), *Usp2*, and *ENSMUSG00000121069* (Fig. 3E). The intersection of these 10 genes and 9 genes from the hub gene key module was taken, yielding 3 potential target genes: *Per1*, *Per2*, and *Per3* (Fig. 3F). *Per1*, *Per2*, and *Per3* are important members of rhythmic processes, and RNA sequencing showed that their expression was upregulated after GA treatment. Based on the above results, *Per1*, *Per2*, and *Per3* play important roles in GA-regulated circadian rhythmic processes.

3.4 GA Upregulates *Per1*, *Per2*, and *Per3* Expression in the Aorta of AS Mice

We first performed serum biochemical and inflammatory marker tests on AS mice. As shown in Fig. 4A–D, compared with the Ctrl group, the levels of LDL-C, TC, and TG in the serum of AS mice were significantly elevated, while the level of HDL-C was significantly reduced. After GA treatment, the levels of LDL-C, TC, and TG were significantly reduced, and HDL-C levels were significantly increased. As shown in Fig. 4E,F, GA significantly inhibited the elevation of IL-6 and TNF- α levels induced by AS. To clarify the effects of GA on *Per1*, *Per2*, and *Per3*, we performed qPCR detection, as shown in Fig. 4G–I. GA significantly promoted the mRNA expression of *Per1*, *Per2*, and *Per3* in the aorta of AS mice, which was consistent with the RNA sequencing results. The above results preliminarily confirmed that the inhibitory effect of GA on AS was related to its regulation of the circadian rhythm genes *Per1*, *Per2*, and *Per3*, and further to the inhibition of inflammation.

4. Discussion

AS is a primary risk factor for cardiovascular diseases, including coronary heart disease, myocardial infarction, and ischemic stroke. Exploring methods to inhibit the progression of AS and reduce its associated disability and mortality rates is of significant importance. In this study, AS mice were treated with GA, and their aortic tissue was subjected to RNA sequencing. This process identified *Per1*, *Per2*, and *Per3* as the target genes through which GA exerts its therapeutic effects. Through a combination of biological experiments and bioinformatics analysis, it was found that GA significantly inhibited AS in mice, with its mechanism of action potentially involving regulation of circadian rhythms by promoting the expression of *Per1*, *Per2*, and *Per3*, thereby inhibiting inflammation.

Molecular clock systems govern biological circadian rhythms in living organisms. These intrinsic clocks are distributed across vascular tissues, peripheral organs, and immune cells. Accumulating evidence strongly supports a link between circadian rhythm dysregulation and disease pathogenesis [17]. A growing body of evidence suggests that an increasing number of Chinese herbal medicine ingredients are being discovered to target rhythms to achieve therapeutic effects. Research has confirmed that berberine, a component of *Coptis chinensis*, can suppress inflammatory expression by targeting the clock component nuclear receptor subfamily 1, group D, member 1 (Rev-erba), thereby treating chronic enteritis [18]. Cycloastragenol increased the levels of clock proteins basic helix-loop-helix ARNT-like 1 (BMAL1) and PER2 in the livers of aged mice, thereby restoring the circadian rhythmic behavior of D-galactose-induced aged mice [19]. Mao et al. [20] found that the Chinese formula Guben-Jiannao Ye enhanced cognitive abilities in amyloid precursor protein

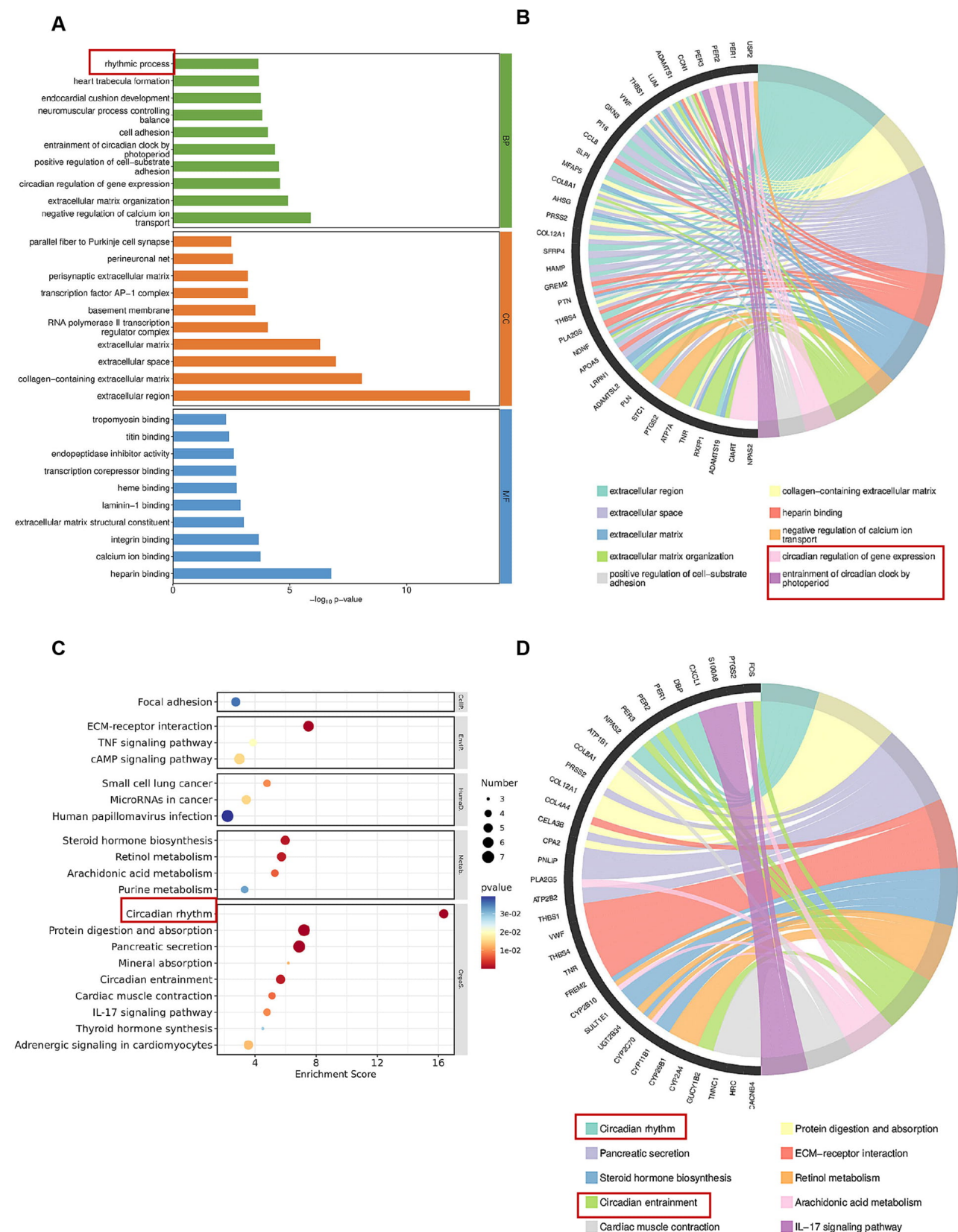


Fig. 2. The effect of GA on pathways and biological processes involving DEGs. (A) GO analysis of biological processes, cellular components, and molecular functions. (B) Co-DEGs involved in biological processes are displayed in a chord diagram. (C) KEGG enrichment analysis. (D) Co-DEGs involved in pathways are shown in a chord diagram. Red boxes indicate significantly enriched circadian rhythm-related terms or pathways. KEGG, Kyoto Encyclopedia of Genes and Genomes.

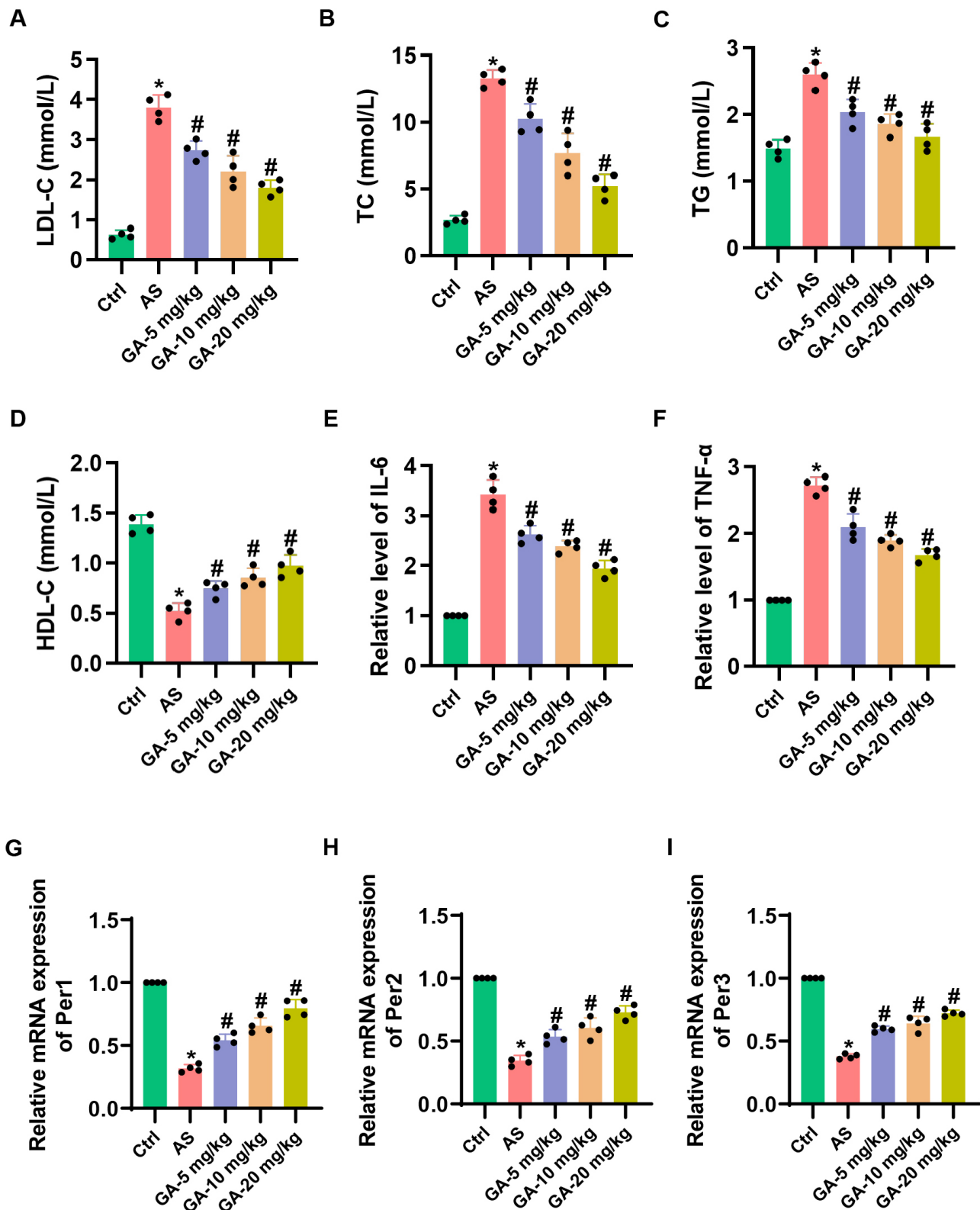


Fig. 4. The effect of GA on target genes. (A–D) Serum biochemical detection of LDL-C, TC, TG, and HDL-C levels in AS mice. (E,F) ELISA detected IL-6 and TNF- α levels. (G–I) qPCR detected the mRNA expression of *Per1*, *Per2*, and *Per3*. * $p < 0.05$ vs. Ctrl. # $p < 0.05$ vs. AS. LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; ELISA, enzyme-linked immunosorbent assay; TNF- α , tumor necrosis factor- α . The data for IL-6 and TNF- α were normalized by dividing the values of each group by the corresponding Ctrl group values. This normalization was applied to all 4 biological replicates before statistical analysis.

(APP)/presenilin-1 (PS1) mice, altered clock gene expression patterns, and alleviated sleep-wake circadian rhythm disorders. In cardiovascular disease, resveratrol has been shown to induce sirtuin 1 (SIRT1) expression, thereby regulating circadian rhythms at the cellular and organismal levels [21]. In this study, we identified differentially expressed genes in GA-treated atherosclerosis using RNA sequencing and performed KEGG and GO enrichment analyses. The results showed that most genes were significantly enriched in circadian rhythm pathways and biological processes. Furthermore, the three target genes *Per1*, *Per2*, and *Per3* were identified as important regulators of circadian rhythms. These findings suggest that the inhibitory effect of GA on atherosclerosis may be related to circadian rhythms.

Per1, *Per2*, and *Per3* are members of the Per (Period) gene family and are core components of the biological circadian rhythm regulatory system. They are widely present in eukaryotic organisms ranging from fruit flies to humans, and their rhythmic fluctuations in expression and function are key to maintaining the stability of the biological clock. Research has confirmed that these 3 genes are widely involved in the progression of cardiovascular disease [22]. Disruption of *Per1* expression exacerbates neurological and brain damage in mouse with ischemic stroke [23]. Another study has found that the mechanism by which melatonin may inhibit water immersion-induced myocardial damage by regulating the expression of circadian clock genes, such as *Per1* [24]. Melatonin has been shown to exert a cardioprotective effect by regulating *Per2* expression and inhibiting myocardial ischemia/reperfusion [25]. *Per3* has also been shown to exhibit significant changes in expression during myocardial hypertrophy, suggesting that it is an important factor influencing the occurrence and progression of hypertrophy [26]. Our research confirms that GA can regulate *Per1*, *Per2*, and *Per3* in the aorta of AS mice. This suggests they may be potential targets for AS treatment.

5. Limitations

This study has several limitations. First, the specific upstream signaling pathways mediating GA-induced upregulation of *Per1*, *Per2*, and *Per3* remain to be elucidated. Second, *in vivo* evidence from ApoE^{-/-} mice lacked cell-level rescue experiments, limiting the ability to establish a direct functional link between GA and these clock genes. Third, the study did not examine the temporal dynamics of circadian gene expression across multiple time points or validate the findings using human clinical samples, thereby limiting the translational relevance.

6. Conclusions

In summary, GA can significantly inhibit AS in mice, which may be achieved by regulating the circadian rhythm genes *Per1*, *Per2*, and *Per3*, thereby attenuating inflammatory responses. This provides new strategies and targets for the treatment of AS.

Availability of Data and Materials

All raw sequencing, biochemical, and qPCR data and original figures are stored locally and not publicly deposited. Data cannot be released openly per Ethics Approval No.2025294 to protect confidential mouse metadata, and these datasets support our unfinished related studies. Complete raw materials will be confidentially shared with editors, reviewers and readers for academic verification via written request to the corresponding author, complying with IMR Press requirements.

Author Contributions

Conceptualization, CH; Methodology and validation, FR, YG, JR, ME; Investigation, FR; Writing—original draft preparation, CH; Writing—review and editing, ME; Supervision, ME; Project administration and funding acquisition, CH, JR, ME. All authors have read and agreed to the published version of the manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal study protocol was approved by the Animal Ethics Committee of Changchun University of Chinese Medicine (Approval No. 2025294). All animal experiments in this study were performed in accordance with the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals (NRC, 8th edition, 2011).

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/Pharmazie55101>.

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