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Ponicidin alleviates atherosclerosis by inhibiting inflammation and oxidative stress through the SIRT1 and PI3K/Akt pathway

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ABSTRACT

Objective(s): Atherosclerosis (AS) is caused by dyslipidemia, chronic inflammation, and oxidative stress. Ponicidin, a diterpenoid from Isodon species, has reported anti-inflammatory and antioxidant effects, but its role in AS remains unclear. In this study, we evaluated the therapeutic potential and safety of ponicidin in an experimental AS model.

Materials and Methods: ApoE-/- mice were fed a Western diet and treated with ponicidin (10 mg/kg/day, intraperitoneally) for 12 weeks. Hepatic and renal safety were assessed using histological and serum biochemical markers. Aortic plaque burden was quantified using hematoxylin and eosin and Oil Red O staining. Plasma lipids, cytokines, oxidative stress markers, and antioxidant enzymes were measured using biochemical assays and ELISA. The effects on SIRT1 and PI3K/Akt/eNOS signaling were analyzed using immunohistochemistry, RT-qPCR, ELISA, and Western blotting.

Results: Ponicidin showed no liver or kidney toxicity and moderately altered the body weight. Treatment significantly reduced aortic root plaque coverage and lesion area while improving plasma lipid profiles (↓TG, ↓TC, ↓LDL-C, and ↑HDL-C). Serum cytokine analysis revealed decreased TNF-α, IL-1β, IL-6, MCP-1, and TGF-β1 levels, with elevated IL-10. Oxidative stress was attenuated, as evidenced by reduced malondialdehyde and myeloperoxidase levels, increased superoxide dismutase, catalase, and glutathione levels, and restored nitric oxide levels. Mechanistically, ponicidin up-regulated SIRT1 expression and enhanced the phosphorylation of PI3K, Akt, and eNOS, suggesting improved endothelial function.

Conclusion: Ponicidin protects against AS by improving lipid metabolism, reducing vascular inflammation and oxidative stress, and enhancing endothelial signaling.

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Introduction

Atherosclerosis, a chronic inflammatory disease of the arterial wall, is the principal pathological underpinning of cardiovascular diseases (CVDs), which are the leading cause of morbidity and mortality worldwide (1). The pathogenesis of atherosclerosis involves a complex interplay between endothelial dysfunction, lipid accumulation, chronic inflammation, and oxidative stress in the subendothelial space. This environment promotes the recruitment of monocytes, their differentiation into macrophages, and the uncontrolled uptake of oxidized low-density lipoprotein (ox-LDL), leading to the formation of foam cells and fatty streaks, which are hallmarks of early atherosclerotic lesions (2).

Two central mechanisms driving this pathology are inflammation and stress. Inflammatory signaling pathways, particularly those mediated by NF-κB, drive the expression of adhesion molecules, cytokines, and chemokines, which perpetuate the inflammatory cycle (3). Concurrently, oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defense, exacerbates endothelial damage, promotes LDL oxidation, and amplifies inflammatory responses (4). Despite advances in therapeutic strategies, such as statin therapy, which primarily target lipid levels, a significant residual inflammatory risk remains, underscoring the urgent need for novel therapies that directly target these core inflammatory and oxidative pathways (5).

Sirtuin 1 (SIRT1), a NAD+-dependent class III histone deacetylase, has emerged as a critical regulator of vascular homeostasis and a potent atheroprotective molecule. SIRT1 deacetylates key targets, including the p65 subunit of NF-κB and the transcription factor FOXO3, thereby suppressing inflammatory gene expression and enhancing antioxidant defense (6, 7). The PI3K/Akt signaling pathway is a crucial modulator of cell survival, metabolism, and oxidative stress. Activation of PI3K/Akt can inhibit apoptosis, promote nitric oxide (NO) production, and activate the antioxidant transcription factor Nrf2, collectively contributing to endothelial protection (8, 9). A growing body of evidence suggests crosstalk between SIRT1 and the PI3K/Akt pathway, which can mutually activate each other to form a

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robust defensive network against cellular stress (10).

Ponicidin, a bioactive diterpenoid primarily isolated from *Rabdosia rubescens* and other Isodon species, exhibits potent anti-inflammatory, antioxidant, and anticancer activities (11-13). The chemical structure, molecular weight, chemical formula, and CAS number are shown in Figure 1A. Recent studies have indicated that ponicidin may exert neuroprotective effects, potentially by modulating key cellular pathways. SIRT1 (sirtuin 1) and PI3K/Akt signaling pathways are critical regulators of cell survival, metabolism, and stress resistance (14, 15). SIRT1, an NAD+-dependent deacetylase, enhances mitochondrial function and mitigates oxidative damage (16), whereas the PI3K/Akt signaling pathway inhibits apoptosis and promotes neuronal survival (17). Given their central roles in controlling oxidative stress and cell survival, the SIRT1 and PI3K/Akt pathways may mediate the neuroprotective effects of ponicidin in Aβ1-42-induced hippocampal injury. Beyond its neuroprotective potential, ponicidin exerts broader pharmacological effects by modulating the signaling pathways involved in metabolic and inflammatory processes. However, its specific role in atherosclerosis and the underlying mechanisms, particularly its interaction with the SIRT1 and PI3K/Akt axes, remain largely unexplored.

This study aimed to evaluate the effects of ponicidin on atherosclerotic progression in ApoE-/- mice, focusing on its effects on systemic inflammation, oxidative stress, and the SIRT1 and PI3K/Akt signaling pathways. We hypothesized that ponicidin attenuates AS by modulating these pathways, thereby reducing vascular inflammation and oxidative injury.

Materials and Methods

Animals

Male C57BL/6 mice (6-8 weeks, 22-24 g) and male apolipoprotein E-deficient (ApoE-/-) mice were purchased from Jiangsu Huachuang Xinnuo Pharmaceutical Technology Co., Ltd. (Jiangsu, China). C57BL/6 mice (n=8) were used as the control group and were fed a maintenance diet (4% fat, 1025, Beijing HFK Bioscience Co., Ltd.). ApoE-/- mice were fed for 12 weeks with western diet (21% fat, 0.15% cholesterol, H10141, Beijing HFK Bioscience Co., Ltd.), and randomly divided into 2 groups (n=8): atherosclerosis model (AS) group and Ponicidin group (10 mg/kg). The Control and AS group mice were intraperitoneally injected with saline for 12 weeks, and the ponicidin group mice were intraperitoneally injected with ponicidin (10 mg/kg/day) for 12 weeks. After the completion of the experiment, the mice were fasted for 12 hr and then sacrificed. Peripheral blood samples and aortas were collected from the mice. This study was approved by the Animal Care and Use Committee of our hospital and conformed to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

Liver and renal function

The mice were intraperitoneally injected with either saline or ponicidin (10 mg/kg/day) for 12 weeks. Blood samples were collected from the tail vein, and the serum was separated. Serum levels of alanine transaminase (ALT; C009-2-1, Nanjing Jiancheng) and aspartate aminotransferase (AST; C010-2-1, Nanjing Jiancheng), creatinine (S0291S, Beyotime), and blood urea nitrogen (BUN; S0574S, Beyotime) levels were measured using a microplate reader (μLISKAN MK3, Thermo). Liver and kidney tissues were subsequently subjected to hematoxylin and eosin (H&E) staining.

Body weight and plasma lipid levels

The body weights of the mice were measured at 4, 8, and 12 weeks after ponicidin administration. The lipid changes in AS mice were analyzed by measuring the concentrations of triglycerides (TG; S0219S, Beyotime), total cholesterol (TC; S0211S, Beyotime), low-density lipoprotein cholesterol (LDL-C; A113-1-1, Nanjing Jiancheng), and high-density lipoprotein cholesterol (HDL-C; A112-1-1, Nanjing Jiancheng) in plasma using Lipid Biochemistry Kits.

Aortic root histological analysis

Mouse aortic tissues were fixed in 4% paraformaldehyde and embedded in paraffin blocks. The sections were stained with hematoxylin and eosin (H&E; C0105S, Beyotime) and Oil Red O (C0157S, Beyotime). Immunohistochemical staining was performed to detect SIRT1 expression.

Enzyme-linked immunosorbent assay (ELISA)

Serum was collected 12 weeks after ponicidin administration and stored at -80 ˚C until analysis, avoiding repeated freeze–thaw cycles. The concentrations of tumor necrosis factor-alpha (TNF-α; MTA00B, R&D Systems), interleukin-1 beta (IL-1β; MLB00C, R&D Systems), interleukin-6 (IL-6; M6000B, R&D Systems), monocyte chemoattractant protein-1 (MCP-1; SEKM-0108, Solarbio, China), transforming growth factor-beta 1 (TGF-β1; SEKM-0035, Solarbio, China), interleukin-10 (IL-10; M1000B, R&D Systems), and sirtuin 1 (SIRT1; ml037854, Shanghai Enzyme-linked Biotechnology) were quantified using commercial ELISA kits according to the manufacturers' instructions.

Measurement of serum oxidative indicators

Serum oxidative stress markers were assessed using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocols. Malondialdehyde (MDA; A003-1-2), myeloperoxidase (MPO; A044-1-1), superoxide dismutase (SOD; A001-3-2), catalase (CAT; A007-1-1), glutathione (GSH; A006-2-1), and nitric oxide (NO; A012-1-2) levels were determined. Briefly, the serum samples were thawed on ice and processed according to the manufacturer's instructions. The absorbance of each assay was measured using a microplate reader (μLISKAN MK3, Thermo), and the concentrations or enzyme activities were calculated based on the standard curves.

Reverse Transcription Quantitative PCR (RT-qPCR)

Total RNA was extracted from mouse tissues using TRIzol reagent (Invitrogen, USA), according to the manufacturer's instructions. The concentration and purity of RNA were determined spectrophotometrically, and 1 μg of RNA was reverse transcribed into cDNA using a reverse transcription kit (Takara, Japan). Quantitative PCR was performed using SYBR Green Master Mix (Takara, Japan) in a real-time PCR detection system (Applied Biosystems, USA). The primer sequences for SIRT1 were as follows: forward, 5′-TCG GCT ACC GAG GTC CAT A-3′; reverse, 5′-AAC AAT CTG CCA

CAG CGT CA-3.' GAPDH was used as an internal control. The relative expression of target genes was calculated using the $2^{-\Delta\Delta Ct}$ method, and all reactions were performed in triplicate to ensure reproducibility.

Western blotting

Total protein was extracted from mouse tissues using RIPA lysis buffer supplemented with protease and phosphatase inhibitors (Beyotime, Shanghai, China). Protein concentrations were quantified using a BCA Protein Assay Kit (Thermo Fisher Scientific, USA). Equal amounts of protein (50 µg) were separated by SDS–PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, USA). Membranes were blocked with 5% non-fat milk in TBST (Tris-buffered saline with 0.1% Tween-20) for 1 h at room temperature and then incubated overnight at 4 ˚C with the following primary antibodies: SIRT1 (1:1000, sc-74465, mouse monoclonal, Santa Cruz), phosphorylated eNOS (p-eNOS, 1:500, ab215717, rabbit monoclonal, Abcam), eNOS (1:500, ab300071, rabbit monoclonal, Abcam), phosphorylated PI3K (p85, Tyr458; 1:400, ab278545, rabbit monoclonal, Abcam), PI3K (1:500, ab302958, rabbit monoclonal, Abcam), phosphorylated Akt (Ser473; 1:400, ab81283, rabbit monoclonal, Abcam), Akt (1:400, ab8805, rabbit polyclonal, Abcam), and GAPDH (1:2000, ab8245, mouse monoclonal, Abcam). After washing with TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (goat anti-mouse or goat anti-rabbit, 1:5000; Jackson ImmunoResearch, USA) for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system (Thermo Fisher Scientific, USA), and band intensities were quantified using ImageJ software. GAPDH was used as an internal loading control.

Statistical analysis

Data are expressed as mean±standard deviation (SD). All statistical analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Differences among three or more groups were assessed using one-way analysis of variance (ANOVA). When significant differences were identified, post hoc comparisons were conducted using the least significant difference (LSD) method. Statistical significance was determined using one-tailed tests, with p-values <0.05 considered significant.

Results

Ponicidin showed no liver or kidney toxicity and altered body weight in AS mice

The chemical structure, molecular weight, and chemical formula of ponicidin are presented in Figure 1A. ApoE-/- mice treated with ponicidin (10 mg/kg/day, intraperitoneally for 12 weeks) showed no histological signs of liver or kidney damage, as indicated by hematoxylin and eosin (HE) staining of the hepatic and renal tissues (Figure B). Serum biochemical markers, including ALT, AST, creatinine, and BUN, were comparable to those of the control group (Figure 1C, D), suggesting that ponicidin does not impair hepatic or renal functions. Body weight measurements at weeks 4, 8, and 12 revealed moderate changes in ponicidin-treated mice compared to control mice (Figure 1E-G). These observations indicate that ponicidin is well tolerated in AS mice and may influence the systemic metabolism. The reduction or modulation of body weight in treated mice could also reflect potential anti-atherosclerotic effects, consistent with the therapeutic profile of ponicidin.

Effects of ponicidin on aortic root plaque, atherosclerotic lesions, and plasma lipid levels

The effects of ponicidin on aortic root plaques, atherosclerotic lesions, and plasma lipid levels were evaluated in ApoE-/- mice fed a Western diet. Representative images of hematoxylin and eosin (HE) and Oil Red O staining of the aortic roots are shown in Figure 2A. Quantitative analysis revealed that ponicidin treatment significantly reduced aortic plaque coverage (Figure 2B), and the percentage of

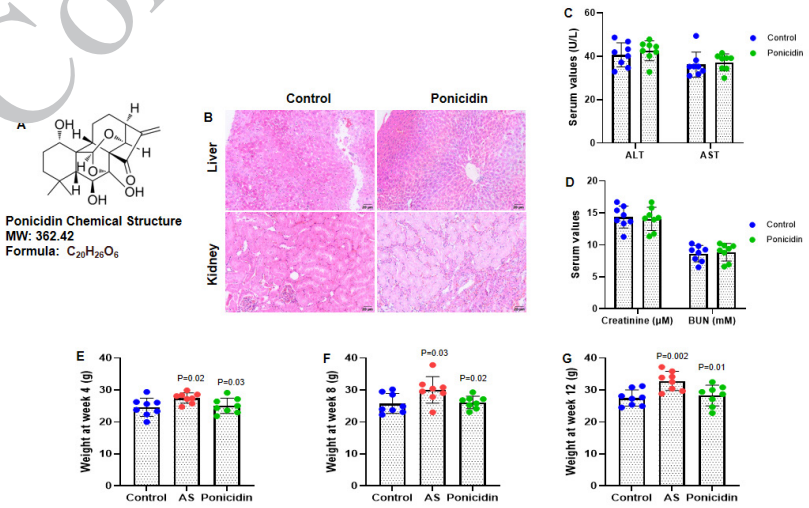

Figure 1. Ponicidin showed no toxicity to the liver and kidneys and did not change the body weight of AS mice

(A) Chemical structure, molecular weight, and chemical formula of ponicidin. (B) Mice were intraperitoneally injected with saline or ponicidin (10 mg/kg/day) for 12 weeks, and the hepatic and renal tissues were stained with hematoxylin and eosin (HE)(magnification 200×). (C, D) Hepatic and renal functions of mice were evaluated by measuring serum ALT, AST, creatinine, and BUN levels. ApoE-/- mice were fed with western diet and intraperitoneally injected with saline or ponicidin (10 mg/kg/day) for continuous 12 weeks, the control mice were fed with maintenance diet and intraperitoneally injected with saline. (E, F, G) Body weights of mice were measured at weeks 4, 8, and 12. Data are presented as mean±SD (n=8 in each group), and analyzed using ANOVA followed by the Bonferroni test. *P<0.05, **P<0.01 vs control group; #P<0.05 vs AS group

AS: Atherosclerosis; ALT: Alanine transaminase; AST: Aspartate transaminase; BUN: Blood urea nitrogen

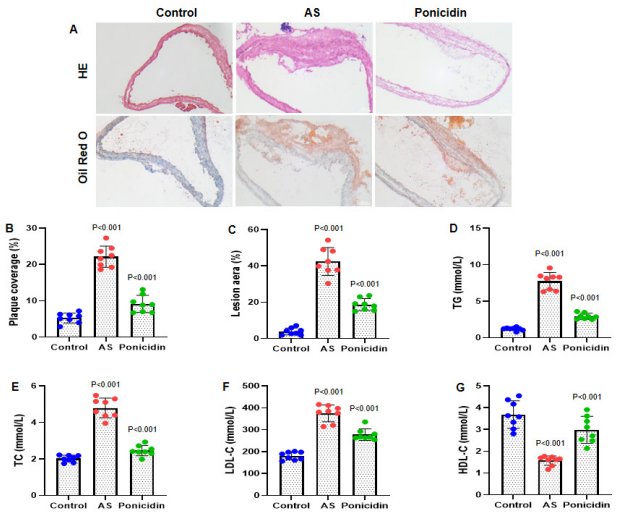

Figure 2. Effects of ponicidin on mouse aortic root plaque, atherosclerotic lesions, and plasma lipid levels
Control group is C57BL/6 mice given maintenance diet, AS model group is ApoE-/- mice given western diet, Ponicidin group is ApoE-/- mice given western diet and intraperitoneal injection with Ponicidin (10 mg/kg/day) for 12 weeks. (A) Representative images of hematoxylin and eosin (H&E) and Oil Red O staining of aortic roots (200×). (B) Quantification of the percentage of plaque coverage by HE staining. (C) Quantification of the percentage of lesion area by Oil Red O staining. Serum levels of (D) TG, (E) TC, (F) LDL-C, and (G) HDL-C were measured in the plasma of mice. Data are presented as mean±SD (n=8 in each group), and analyzed using ANOVA followed by the Bonferroni test. ***P<0.001 vs control group; ###P<0.001 vs AS group

the lesion area (Figure 2C) compared with untreated AS model mice, indicating the inhibition of atherosclerotic progression. Assessment of plasma lipid profiles showed that ponicidin administration decreased serum triglyceride (TG; Figure 2D), total cholesterol (TC; Figure 2E), and low-density lipoprotein cholesterol (LDL-C; Figure 2F) levels, whereas high-density lipoprotein cholesterol (HDL-C; Figure 2G) levels were modestly increased compared to the AS model group. These findings suggest that ponicidin exerts anti-atherosclerotic effects by reducing plaque formation and improving the plasma lipid profile in AS mice. The reduction in LDL-C and TC, along with a trend toward increased HDL-C, may contribute to decreased lipid deposition in the arterial wall, highlighting the therapeutic potential of ponicidin in atherosclerosis.

Effects of ponicidin on systematic inflammatory response in AS mice

The effects of ponicidin on systemic inflammatory responses were assessed in AS mice by measuring serum cytokine levels using ELISA. Compared to the AS model group, ponicidin-treated mice exhibited significantly reduced levels of pro-inflammatory cytokines, including TNF-α (Figure 3A) and IL-1β (Figure 3B), IL-6 (Figure 3C), and MCP-1 (Figure 3D). Additionally, TGF-β1 (Figure 3E), a cytokine involved in fibrosis and chronic inflammation, decreased in the ponicidin group. In contrast, the anti-inflammatory cytokine IL-10 (Figure 3F) was elevated following treatment with ponicidin. These results indicate that ponicidin modulates systemic inflammation in AS mice by suppressing pro-inflammatory mediators and enhancing anti-inflammatory responses. The observed shift in cytokine balance suggests that ponicidin may attenuate chronic vascular inflammation, a key contributor to atherosclerosis

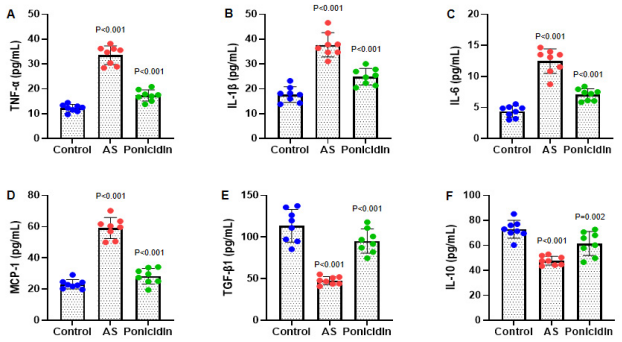

Figure 3. Effects of ponicidin on systemic inflammatory response in atherosclerosis (AS) mice
(A) ELISA was performed to measure the serum levels of (A) TNF-α, (B) IL-1β, (C) IL-6, (D) MCP-1, (E) TGF-β1, and (F) IL-10 in the mice. Data are presented as mean±SD (n=8 in each group), and analyzed using ANOVA followed by the Bonferroni test. ***P<0.001 vs control group; ##P<0.01, ###P<0.001 vs AS group

progression, thereby supporting its potential therapeutic role in AS.

Effects of ponicidin on oxidative stress index in AS mice

The effects of ponicidin on oxidative stress were evaluated in AS mice by measuring the serum levels of key oxidative stress markers. Compared to the AS model group, ponicidin-treated mice exhibited significantly reduced malondialdehyde (MDA; Figure 4A) and myeloperoxidase (MPO; Figure 4B) levels, indicating decreased lipid peroxidation and neutrophil-mediated oxidative activity. Conversely, antioxidant markers, including superoxide dismutase (SOD; Figure 4C), catalase (CAT; Figure 4D), and glutathione (GSH; Figure 4E), were elevated following ponicidin treatment, suggesting enhanced systemic antioxidant capacity. Nitric oxide (NO) levels (Figure 4F) were restored to normal, indicating improved endothelial function. These findings suggest that ponicidin attenuates oxidative stress in AS mice by reducing pro-oxidant activity and enhancing antioxidant defense. The modulation of oxidative stress markers implies that ponicidin may protect vascular tissues from oxidative damage, a key contributor to atherosclerosis progression, thereby reinforcing its potential therapeutic role in AS.

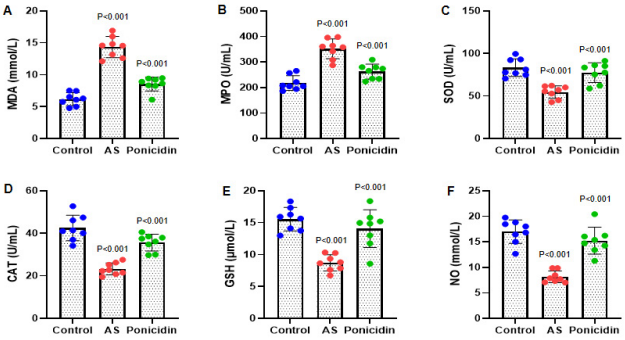

Figure 4. Effects of Ponicidin on oxidative stress index in AS mice
Serum was collected to assess oxidative stress levels. (A) Serum MDA levels. (B) Serum MPO levels. (C) Serum SOD levels. (D) Serum CAT levels. (E) Serum GSH levels. (F) Serum NO levels. Data are presented as mean±SD (n=8 in each group), and analyzed using ANOVA followed by the Bonferroni test. ***P<0.001 vs control group; ###P<0.001 vs AS group
AS: Atherosclerosis; MDA: Malondialdehyde; MPO: Myeloperoxidase; SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione; NO: Nitric oxide

Ponicidin modulates SIRT1, eNOS and the PI3K/Akt pathway in AS mice

The effects of ponicidin on SIRT1, eNOS, and the PI3K/Akt signaling pathways were assessed in AS mice. Immunohistochemistry revealed increased SIRT1 expression in the vascular tissues of ponicidin-treated mice compared to that in the AS model group (Figure 5A). Consistently, serum SIRT1 levels measured using ELISA (Figure 5B) and SIRT1 mRNA expression was determined using RT-qPCR (Figure 5C) were significantly elevated following ponicidin administration. Western blot analysis showed that ponicidin treatment enhanced the protein expression of SIRT1 (Figures 5D and E) and increased eNOS phosphorylation at Ser1177 (Figure 5F), and PI3K at Tyr458 (Figure 5G), and Akt at Ser473 (Figure 5H), indicating activation of the PI3K/Akt/eNOS signaling pathway. These findings suggest that ponicidin up-regulates SIRT1 expression and activates the PI3K/Akt/eNOS pathway in AS mice. The enhancement of SIRT1 may contribute to the anti-inflammatory and antioxidant effects, whereas the activation of eNOS and PI3K/Akt signaling likely promotes endothelial function and vascular protection, highlighting a potential mechanistic basis for the anti-atherosclerotic effects of ponicidin.

Discussion

In this study, we demonstrated that ponicidin (10 mg/kg/day, IP, for 12 weeks) was well tolerated in ApoE-/- mice and exerted potent anti-atherosclerotic effects. Ponicidin treatment markedly reduced the aortic root plaque and lesion areas and improved the plasma lipid profile, as evidenced by decreased TG, TC, and LDL-C levels, with a trend toward increased HDL-C. Ponicidin also suppressed systemic pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, MCP-1, TGF-β1) while enhancing the anti-inflammatory cytokine IL-10. Moreover, it alleviated oxidative stress by lowering the MDA and MPO levels, increasing the SOD, CAT, and GSH levels, and restoring the NO levels. Mechanistically, ponicidin up-regulated SIRT1 expression and promoted the phosphorylation of PI3K, Akt, and eNOS. Together, these complementary actions suggest a multimodal protective mechanism in which ponicidin improves lipid metabolism and stabilizes the vascular microenvironment by attenuating inflammation and oxidative stress, while restoring endothelial signaling.

Histology and serum biochemistry showed no hepatic or renal toxicity after chronic ponicidin treatment, consistent with previous preclinical reports that ponicidin has an acceptable safety profile in rodent disease models (e.g., streptozotocin-induced diabetic nephropathy and myocardial injury models)(18, 19). The modest changes in body weight we observed may reflect the systemic metabolic effects of ponicidin (reduced adiposity or altered lipid metabolism) or simply an improved health status in animals with a lower atherosclerotic burden. Similar body weight modulation has been reported in other cardiometabolic models treated with ponicidin or related diterpenoids (19, 20).

The reduction in plaque burden and improved lipid profile observed in this study aligns with the emerging literature showing that plant-derived polyphenols and diterpenoids can both lower circulating atherogenic lipids and reduce plaque formation in hyperlipidemia models (21). Ponicidin has been reported to ameliorate hyperlipidemia and organ injury in metabolic disease models, and our data extend these findings by demonstrating direct reductions in aortic plaque area in ApoE-/- mice fed a Western diet (18, 19). The lipid-lowering effects (notably reductions in LDL-C and TC) likely contributed to the reduced lipid deposition in the arterial wall and, therefore, to the smaller lesion areas measured.

Ponicidin shifted the systemic cytokine balance toward an anti-inflammatory profile (↓TNF-α, IL-1β, IL-6, MCP-1, TGF-β1; ↑IL-10) while simultaneously reducing

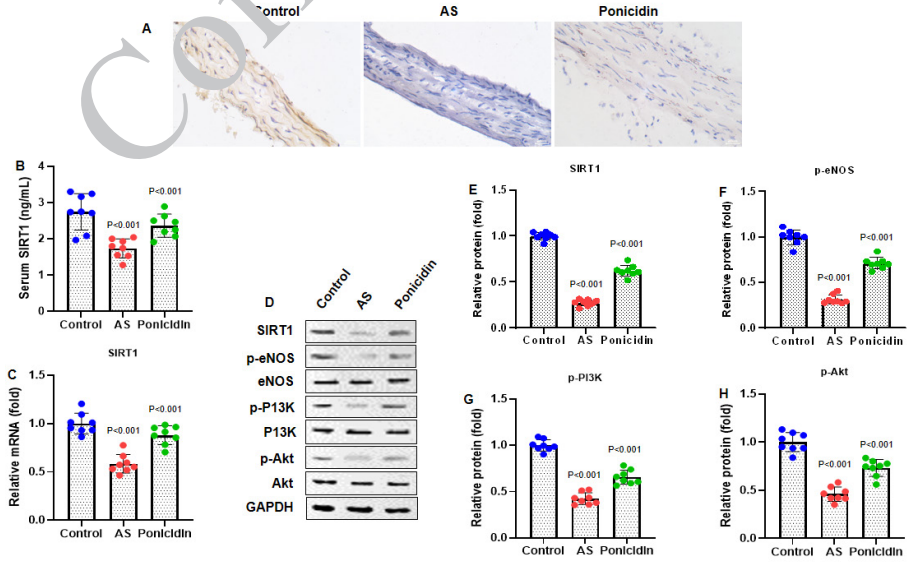

Figure 5. Ponicidin modulates SIRT1, eNOS, and the PI3K/Akt pathway in atherosclerosis (AS) mice
(A) Representative images of SIRT1 expression by immunohistochemistry (200× magnification). (B) ELISA was performed to measure serum SIRT1 levels. (C) RT-qPCR was performed to determine the mRNA expression of SIRT1. (D) Representative gel blots of SIRT1, eNOS, PI3K, and Akt proteins by western blotting. These gel blots were quantified for (E) SIRT1 (normalized to GAPDH), (F) p-eNOS (Ser1177, normalized to total eNOS), (G) p-PI3K (Tyr458, normalized to total PI3K), and (H) p-Akt (Ser473, normalized to total Akt). Data are presented as mean±SD (n=8 in each group), and analyzed using ANOVA followed by the Bonferroni test. ***P<0.001 vs control group; ###P<0.001 vs AS group

oxidative stress markers and enhancing antioxidant defenses. These effects are consistent with earlier studies demonstrating that ponicidin mitigates inflammatory and oxidative injury in models of diabetic nephropathy, including streptozotocin-induced disease, marked by a reduction in pro-inflammatory cytokines and oxidative stress in the renal tissue (19). Comparable effects have been documented for statins: beyond lipid lowering, they exert pleiotropic anti-inflammatory and antioxidant benefits by modulating endothelial function, stabilizing plaques, and suppressing inflammatory mediators such as MCP-1, even in the absence of cholesterol reduction (22). Similarly, natural compounds, such as resveratrol, improve vascular function via mechanisms involving enhanced endothelial NO production, activation of SIRT1, and PI3K/Akt/eNOS signaling pathways (23-25). Given that the suppression of inflammation and oxidative stress is central to atheroprotection, the dual anti-inflammatory and antioxidant profiles of ponicidin offer a plausible mechanistic foundation for its anti-atherosclerotic actions observed in the present study.

A central mechanistic insight from our data is that ponicidin up-regulates SIRT1 expression (IHC, ELISA, RT-qPCR, and WB) and activates the PI3K/Akt/eNOS axis (increased p-PI3K, p-Akt, and p-eNOS Ser1177). This pattern provides a plausible unified mechanism: SIRT1 activation is known to exert anti-inflammatory and antioxidant effects and improve endothelial function, and previous studies have linked SIRT1 activity to enhanced PI3K/Akt/eNOS signaling and NO production in endothelial and progenitor cells (26). Recent studies on ponicidin in neuronal/hippocampal models similarly reported SIRT1 up-regulation and PI3K/Akt activation as key mediators of cytoprotection, supporting the notion that the SIRT1–PI3K/Akt pathway is a conserved downstream pathway for ponicidin across tissues (18). Taken together, our data and these reports suggest that the vascular benefits of ponicidin result, at least in part, from SIRT1 induction, downstream activation of PI3K/Akt, phosphorylation/activation of eNOS, and consequent restoration of NO bioavailability, with secondary anti-inflammatory and antioxidant effects.

Limitations

However, several limitations temper the interpretation of these findings. This study did not include loss-of-function experiments to directly confirm the causal involvement of SIRT1 and PI3K/Akt/eNOS signaling in ponicidin-mediated atheroprotection. Although our data demonstrate a correlated up-regulation of SIRT1 and activation of the PI3K/Akt/eNOS pathway, mechanistic conclusions are based on associative evidence. Formal blockade experiments using SIRT1 inhibitors (e.g., EX-527), PI3K/Akt inhibitors, or genetic approaches are required to establish pathway dependence, as applied in recent ponicidin studies (18). In addition, the use of a single dose (10 mg/kg) and intraperitoneal administration limits the definition of a therapeutic window and oral bioavailability; dose–response and oral dosing studies would enhance translational relevance. Furthermore, although systemic markers and plaque area were assessed, detailed analyses of plaque composition and stability, including macrophage content, necrotic core size, fibrous cap thickness, and collagen content, were not performed and are important determinants of clinical risk. Finally, long-term safety and pharmacokinetic evaluations in additional models, such as LDLR$^{-/-}$ mice or models of metabolic syndrome, are warranted to strengthen the translatability of these findings.

Conclusion

Collectively, our results support a model in which ponicidin is well tolerated in ApoE-/- mice and reduces atherosclerotic burden by improving plasma lipids, suppressing systemic inflammation, reducing oxidative stress, and restoring endothelial PI3K/Akt/eNOS signaling, effects that are plausibly mediated through the up-regulation of SIRT1 (Figure 6). These findings extend prior reports of ponicidin's anti-inflammatory and cytoprotective activities in metabolic and neuroinflammatory models1-3 and suggest ponicidin as a promising natural compound for further preclinical development in atherosclerosis and vascular diseases.

Acknowledgment

Not applicable.

Ethical Approval

This study protocol was reviewed and approved by the Ethics Committee of Guangming Traditional Chinese Medicine Hospital of Pudong New Area (approval number: 2025-KY-002). The authors envisaged all standard protocols in accordance with the 1964 Declaration of Helsinki. All methods carried out in this study were in accordance with ARRIVE guidelines.

Availability of Data and Materials

The data from this study is available upon reasonable request from Dr. Zhongsheng Zhu, at drzzs66@163.com.

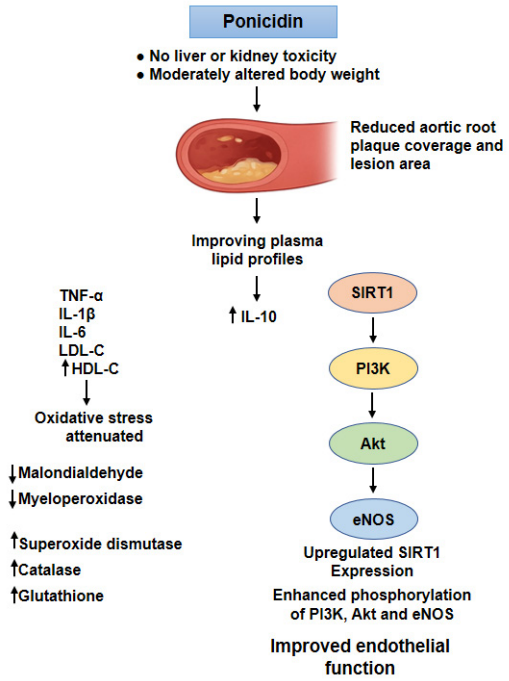

Figure 6. Proposed mechanism of ponicidin in alleviating atherosclerosis in mice
Ponicidin improved lipid profiles, reduced inflammation and oxidative stress, and activated the SIRT1/PI3K/Akt/eNOS pathway, leading to improved endothelial function and reduced atherosclerotic lesion formation in mice

Authors' Contributions

K Y contributed to conceptualization, methodology, investigation, formal analysis, and data curation. J S and P G helped with conceptualization, methodology, investigation, and formal analysis. Z Z contributed to conceptualization, methodology, and supervision. All authors contributed to the writing of this manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Declaration

We have not used any AI tools or technologies to prepare this manuscript.

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