

Relaxation Effect of Schisandrol A on Isolated Thoracic Aorta and its Mechanism in Rats

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Abstract

Background: *Schisandra chinensis* (*S. chinensis*) is a drug commonly used in the clinical treatment of cardiovascular diseases in Traditional Chinese Medicine. However, the specific components and mechanisms of its action are still unclear. We screened six kinds of lignans from *S. chinensis* with high content and found that schisandrol A and schisantherin A had a strong vasorelaxant effect. The purpose of this study was to investigate the relaxation and underlying mechanism of schisandrol A in the isolated thoracic aorta of rats.

Materials and Methods: Isolated rat endothelium-intact and endothelium-removed thoracic aorta strips were pre-constricted with phenylephrine (PE), and the relaxation of schisandrol A on the strips was observed. Then, the mechanism was explored by pre-incubating the strips with nitric oxide synthetase inhibitor N ω -nitro-L-arginine methyl ester (L-NAME), cyclooxygenase inhibitor (indomethacin), potassium channel blockers 4-aminopyridine (4-AP), barium chloride (BaCl₂), tetraethylamine (TEA), and glibenclamide, respectively, and changing the calcium concentration in the bath. In addition, expressions of endothelial nitric oxide synthetase (eNOS) mRNA and protein in rat thoracic aorta were detected.

Results: Schisandrol A induced both endothelium-dependent and endothelium-independent relaxation of isolated thoracic aorta strips of rats, and the mechanism might be related to promoting the synthesis of NO, inhibiting Ca²⁺ release from the sarcoplasmic reticulum, and blocking the Ca²⁺ channels.

Conclusion: These discoveries may provide a theoretical basis for the traditional application of *S. chinensis* to treat cardiovascular disease.

Keywords

Schisandra chinensis, schisandrol A, rat thoracic aorta, vasorelaxation, endothelium

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Key Messages

Schisandrol A induced both endothelium-dependent and independent vasorelaxation of isolated rat thoracic aorta. The endothelium-dependent vasorelaxation might be related to activating eNOS to promote NO synthesis, while endothelium-independent vasorelaxation might be related to blocking calcium channel opening.

such as lowering blood pressure, modulating blood lipids, anti-atherosclerosis, anti-oxidation, relaxing blood vessels, and so on (Demirel, 2022a, 2022b; Jin et al., 2023; Liu et al., 2023).

Introduction

Cardiovascular disease (CVD) has been considered one of the most common diseases threatening human health worldwide since the 21st century (Cheng et al., 2023). A variety of traditional Chinese medicines and their active ingredients have achieved good therapeutic effects in cardiovascular diseases,

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In this regard, *Schisandra chinensis* (Turcz.) Baill. (*S. chinensis*), a genuine medicinal material of Changbai Mountain in China, was first recorded in Shennong's Herbal Classic. *S. chinensis* is often used to relieve hypertension, coronary heart disease, and heart failure in clinics (Ko et al., 1995; Li et al., 1996; Zhaoxu et al., 2016). In Korea, *S. chinensis* is used to treat cardiovascular symptoms in menopause syndrome in women (Chen & Li, 1993). However, there are few studies on the material basis of *S. chinensis* in the treatment of cardiovascular diseases, and its mechanism of action is unclear. Rhyu et al. (2006) reported that the aqueous extract of *S. chinensis* could relax the isolated thoracic aorta of rats. Park et al. (2009) found that the hexane extract dilated the rat thoracic aorta concentration-dependently. Our previous study demonstrated that *S. chinensis* total lignans could significantly relax phenylephrine (PE) pre-contracted isolated thoracic aorta of rats. The above findings suggest that the therapeutic effects of *S. chinensis* on cardiovascular diseases may be related to its vasodilation. In order to further explore the specific components of *S. chinensis*, we screened six *S. chinensis* lignans with higher contents, of which schisandrol A and schisantherin A were found to have a strong relaxing effect on isolated rat vascular strips.

Schisandrol A, a biphenyl cyclooctene lignan with a molecular weight of 432.51, is a lignan monomer with a high content in *S. chinensis* (Figure 1). Schisandrol A is taken as an index component for evaluation of the quality of *S. chinensis* in Pharmacopoeia of the People's Republic of China, and its content is stipulated not less than 0.4% in *S. chinensis* (Chinese Pharmacopoeia Commission, 2015). To further study the vasorelaxation effect of schisandrol A and its mechanism, a vascular tension detection experiment *in vitro* was employed, and a variety of tool agents such as nitric oxide synthase inhibitor, cyclooxygenase inhibitor, and potassium channel blockers were used to speculate its mechanism. This study was expected to give pharmacological evidence for the research and development of *S. chinensis*.

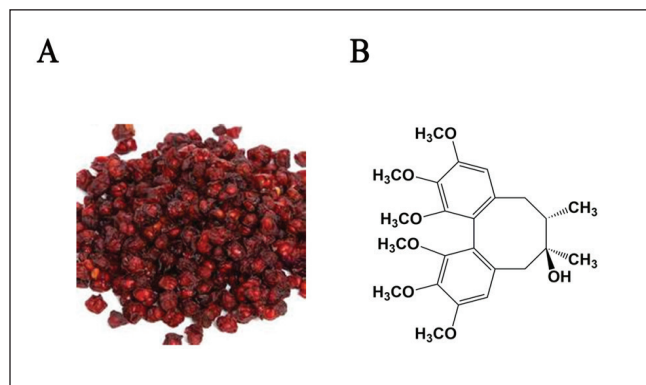


Figure 1. (A) *Schisandra chinensis* (Turcz.) Baill. (B) The Chemical Structure of Schisandrol A.

Materials and Methods

Chemicals and Reagents

Schisandrol A (No. wkq18022702) was purchased from Chengdu Pufuide Biotech Co., Ltd. (Sichuan, China). Phenylephrine, L-NAME, indomethacin, glibenclamide, 4-aminopyridine (4-AP), and barium chloride (BaCl₂) were obtained from Sigma Aldrich (St. Louis, MO, USA). ACh was supplied by Sinopharm Chemical Reagent Co., Ltd., (Beijing, China). A polyclonal antibody of endothelial nitric oxide synthetase (eNOS) was bought from ABClonal Biotechnology (Wuhan, China). Schisandrol A was dissolved with DMSO, and the final concentration of DMSO was controlled within 0.1% (v/v).

Animal Handling

Male SD rats (250–300 g) were purchased from Changchun Yisi Experimental Animal Technology Co., Ltd. [License No.: SCXK (Ji) 2018-0007] and fed in a laboratory under conditions of $20 \pm 2^\circ\text{C}$ and $50 \pm 10\%$ relative humidity. The rats were permitted to eat and drink freely. Adapting feeding for 1 week, rats could be used for the experiment.

Preparation of Isolated Rat Thoracic Aorta Strips

The preparation process referred to our previous report (Yang et al., 2020). In brief, rats were anesthetized with 100 mg/kg urethane, and then the thoracic aorta was quickly cut and placed in a pre-cooled (4°C) modified Ringer-Locke solution (Demirel, 2022). After carefully removing the surrounding tissues, the vascular strips about 3 mm in width and 0.8 cm in length were prepared by helical cutting. The endothelial tissue of some strips was gently wiped off with a small cotton ball to prepare endothelium-denuded vascular strips for observing the endothelium-independent relaxing effect. Subsequently, each specimen was vertically installed in a 10-mL bath tube containing nutrient solution with pH 7.4, in which the temperature was constantly kept at 37.0°C , and 95% O₂ and 5% CO₂ were aerated. Resting tension should be adjusted to 1.5 g to obtain a maximal contraction. The specimen was equilibrated at least 30 min before the beginning of the formal experiment, and the fresh solution was replaced every 10 min.

After the stabilizing of specimen tension, 30 mM KCl solution was used for pre-stimulating the strip twice to activate them. Then, phenylephrine (10^{-6} M) was added into the bath to constrict the strip after its tension was stabilized again, and ACh (10^{-9} – 10^{-5} M) was applied after its tension reached the peak to detect the integrity of vascular endothelium. If the maximum relaxation rate induced by ACh was greater than 90%, the specimen was considered with an intact endothelium, and if less than 10%, without endothelium. Papaverine (10^{-4} M) was applied to produce the maximum diastolic response as a reference. Then, the relaxation induced

by the experimental drug was calculated as a percentage relative to the relaxation induced by papaverine.

Effect of Schisandrol A on PE-pre-contracted Isolated Rat Thoracic Aorta

Schisandrol A at a cumulative concentration of 10^{-7} – 10^{-3} M was successively added to the bath tube after the aortic strip was pre-contracted by 10^{-6} M phenylephrine, and the tension changes were recorded for investigating vasodilation of schisandrol A on endothelium-intact and -denuded aortic strips. The percentage of vasodilation induced by schisandrol A was computed according to the following formula: (maximal tension by phenylephrine – minimal tension by schisandrol A)/(maximal tension by phenylephrine – minimal tension by PA) $\times$ 100% (Yang et al., 2020). Finally, the dose–response curve was drawn based on the relaxation rate of the aortic strips.

Blocking NO and PGI₂ Production

The aortic strip with intact endothelium was pre-incubated with 10^{-4} M L-NAME or 10^{-5} M indomethacin for 10 min to investigate whether endothelium-derived NO and PGI₂ pathways could be involved in the vasorelaxation induced by schisandrol A. Then, 10^{-6} M phenylephrine was applied to induce pre-contraction. After the tension of the strip was stable, schisandrol A at a cumulative concentration of 10^{-7} – 10^{-3} M was added into the bath tube to observe the vasorelaxation.

Blocking Four Types of Potassium Channels

It was unclear whether potassium channel activation was involved in the vasorelaxation induced by schisandrol A. Therefore, four potassium channel blockers, namely, 10^{-3} M BaCl₂ for inward rectifier potassium channel (K_{IR}), 10^{-3} M 4-AP for voltage-dependent potassium channel (K_V), 10^{-3} M TEA for calcium-activated potassium channel (K_{Ca2+}), and 10^{-6} M glibenclamide for ATP-sensitive potassium channel (K_{ATP}), were pre-incubated with endothelium-denuded aortic strips for 20 min, respectively. Then, phenylephrine was used to pre-contrast the strips. Schisandrol A at a cumulative concentration of 10^{-7} – 10^{-3} M or the vehicle was added to the bath tube after the strip tension was stable.

Intracellular Calcium Release Test

Intracellular calcium is involved in the contraction of smooth muscle, and phenylephrine activates the α -receptor, promotes the release of calcium ions from the sarcoplasmic reticulum (SR), and then induces muscle contraction. The present experiment was performed to investigate whether schisandrol A could cause the Ca²⁺ release from SR. Vascular strips without endothelium were incubated in Ca²⁺-free nutrient solution (containing 10^{-3} M EGTA) for 30 min, and then 10^{-6} M phenylephrine was added into the bath to induce the first transient constriction (T₁). Subsequently, the strips

were washed with Ca²⁺-containing nutrient solution three times to supplement the intracellular Ca²⁺ loss and with Ca²⁺-free nutrient solution twice in succession (Yang et al., 2020). Then, schisandrol A (10^{-3} M) was applied and incubated for 15 min, and the second transient vasoconstriction (T₂) in strips was induced through 10^{-6} M phenylephrine. The value (T₁–T₂) was calculated as the tension difference.

Extracellular Calcium Influx Observation

Endothelium-removed vascular strips were incubated in Ca²⁺-free nutrient solution (containing 10^{-3} M EGTA). Notably, 10^{-6} M phenylephrine or 60 mM KCl was used to induce a basic constriction, and then 10^{-6} – 10^{-2} M CaCl₂ was applied in turn to draw a concentration-response curve. The relationship between the vasorelaxation induced by schisandrol A and the vasocontraction induced by phenylephrine or KCl was observed. The specimen was pre-incubated with 10^{-3} M schisandrol A for 15 min before using phenylephrine or KCl. The vasocontraction induced by the highest concentration of CaCl₂ (10^{-2} M) was taken as 100%, and the inhibitory rate of schisandrol A could be calculated based on it.

Total RNA Preparation and RT-PCR

Total RNA was prepared, and RT-PCR was performed as described in our previous study (Yang et al., 2020). In brief, the endothelium-intact aortic segment was pre-incubated with 10^{-3} M schisandrol A at 37°C for 15 min first, and then total RNA was extracted through the use of Trizol reagent, reverse transcribed, and amplified by following the instructions of HiScript II One-Step RT-PCR Kit. Specific primer sequences for rat eNOS were as follows: forward: 5'-CGCCTACCCAAGAAACACCT-3' and reverse: 5'-GCTGACTCCCTCCCAGTCTA-3', and the amplification conditions were denaturation at 94°C, annealing at 56°C, and extension at 72°C for 30 s, with 30 cycles. To confirm the PCR results, the agarose gel electrophoresis on 5 μ L of PCR products was performed at 100 V for 40 min. The images of the results were observed and analyzed by the Tanon 1600 Gel Image system (Shanghai Tianneng Tech. Co. Ltd., China).

Western Blot Detection

The endothelium-intact aortic segment was pre-incubated with 10^{-3} M schisandrol A at 37°C for 15 min, and then total proteins were obtained by using Trizol reagent (Beytime Biotechnology, Shanghai, China). The protein concentration of the sample was measured by the BCA method, separated by SDS-polyacrylamide gel electrophoresis, and transferred onto the PVDF membrane, and then the membrane was blocked with 5% skim milk powder for 1.5 h. Subsequently, the membranes were incubated with the primary antibodies p-eNOS (1:1000 dilution) and GAPDH (1:20000 dilution), respectively, at 4°C overnight. After washing three times, the membranes were incubated with the secondary antibody

routinely in a shaker at room temperature for 1 h. The images were acquired by chemiluminescence, visualized by autoradiography, and quantified densitometrically (Image J). Values for p-eNOS were normalized to GADPH.

Statistical Analysis

Statistical analysis was performed by use of SPSS software (Windows 22.0 version). All data were shown as mean $\pm$ SD (standard deviation). The statistical significance between the two groups was compared by Students' *t*-test, and that of multiple comparisons was analyzed using a one-way ANOVA. A value with $p < 0.05$ was considered a significant difference in statistics.

Results

Relaxation of Schisandrol A on PE-pre-contracted Isolated Rat Thoracic Aorta Strips

Schisandrol A (10^{-7} – 10^{-3} M) could induce a remarkable and concentration-dependent relaxation of both endothelium-intact and endothelium-removed aortic strips pre-contracted with phenylephrine. The maximum relaxation rates reached $52.79 \pm 1.43\%$ and $25.18 \pm 6.83\%$ for schisandrol A in comparison with $2.65 \pm 1.53\%$ and $3.47 \pm 2.25\%$ for the control (Figure 2), indicating that endothelium might be partly involved in the vasorelaxation induced by schisandrol A.

Effects of Blocking NO and PGI₂ Production on Schisandrol A-induced Vasorelaxation

As shown in Figure 3, pre-incubation with L-NAME could obviously reduce the relaxation induced by schisandrol A with $E_{\max}$ of $33.24 \pm 1.04\%$, compared with the control group with $E_{\max}$ of $52.79 \pm 3.27\%$ ($p < 0.001$). However, pre-incubation with indomethacin had no significant effect on vasorelaxation by schisandrol A. These suggested that the endothelium-dependent vasodilation of schisandrol A might be mainly related to the increase of NO synthesis, but not the PGI₂ synthesis.

Effects of Four K⁺ Channel Blockers on Schisandrol A-induced Vasorelaxation

In order to clarify whether the K⁺ channel would be involved in the regulation of vasodilation induced by schisandrol A, four kinds of K⁺ channel blockers, BaCl₂, TEA, 4-AP, and glibenclamide were incubated with endothelium-removed thoracic aorta strips, respectively. The results showed that none of the four K⁺ channel blockers produced an effect on schisandrol A-induced vasorelaxation (Figure 4), indicating that the potassium channel or currents might not be involved in this relaxation.

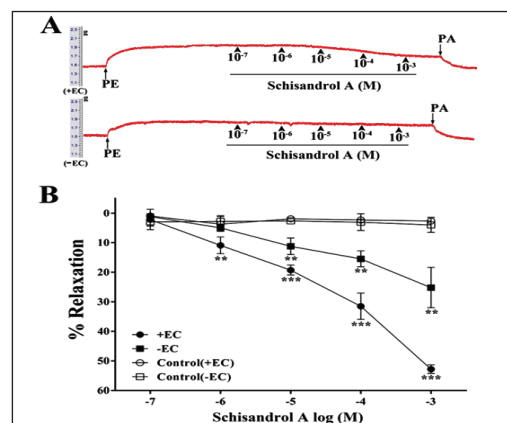


Figure 2. Effects of Schisandrol A on the Tension of Isolated Rat Thoracic Aorta Strips Pre-contracted by PE. (A) Typical Tracings of the Responses About Schisandrol A on the Strips. (B) Cumulative Concentration-response Curves of Schisandrol A on Endothelium-intact (+EC) and Endothelium-denuded (-EC) Aortic Strips, Pre-contracted with PE (10^{-6} M).

Note: The data are shown as the means $\pm$ SD ($n = 6$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ vs. control group).

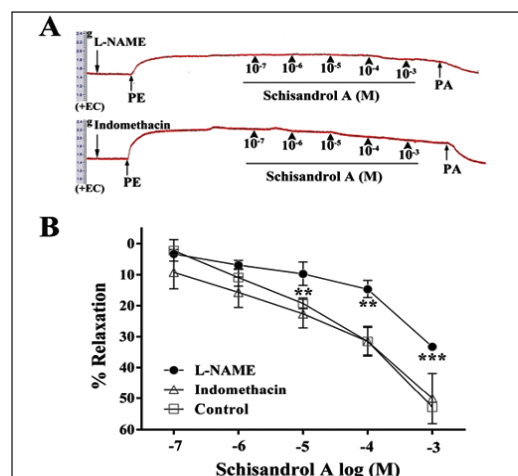


Figure 3. Effects of L-NAME and Indomethacin on the Vasorelaxation Induced by schisandrol A. (A) Typical Tracings of the Responses About Schisandrol A on the Strips Pre-incubated With L-NAME or Indomethacin. (B) Cumulative Concentration-response Curves of Schisandrol A in Endothelium-intact Strips Pre-contracted with PE in the Presence or Absence of L-NAME (10^{-4} M) or Indomethacin (10^{-5} M).

Note: The data are shown as the means $\pm$ SD ($n = 6$, $*p < 0.01$, $***p < 0.001$ vs. control group).

Effect of the Calcium Release from SR on Schisandrol A-induced Vasorelaxation

To elucidate if the vasorelaxation produced by schisandrol A would be related to the inhibition of Ca²⁺ release from SR of

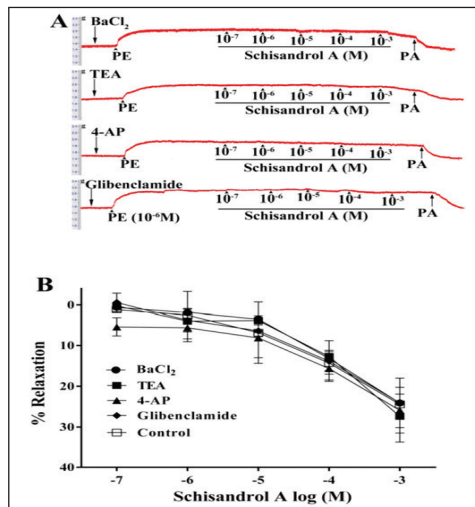


Figure 4. Effects of K⁺ Channel Blockers on the Schisandrol A-induced Vasorelaxation. (A) Typical Tracings of the Responses About Schisandrol A on the Strips Pre-incubated with K⁺ Channel Blockers. (B) The Relaxations of Aortic Strips Pre-incubated With BaCl₂ (10⁻³ M), TEA (10⁻³ M), 4-AP (10⁻³ M), and Glibenclamide (10⁻⁶ M).

Note: The data are shown as the means $\pm$ SD ($n = 6$).

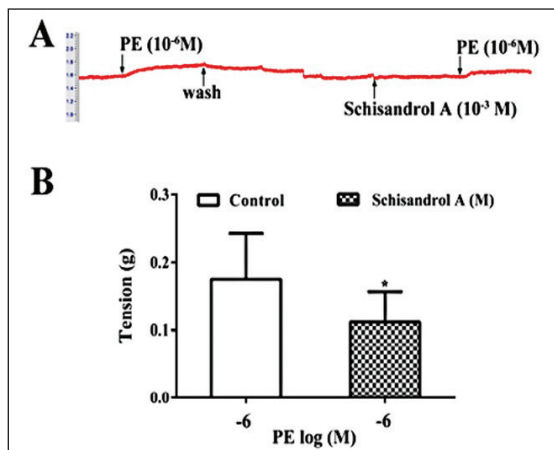


Figure 5. Effect of the Calcium Release from SR on the Schisandrol A-induced Vasorelaxation. (A) Typical Tracings of the Responses About Schisandrol A on the Strips Stimulated by 10⁻⁶ M PE Under a Ca²⁺-free Condition. (B) The Inhibitory Effects of Schisandrol A (10⁻³ M) on Endothelium-denuded Aortic Strips-induced Contraction by PE (10⁻⁶ M).

Note: The data are shown as the means $\pm$ SD ($n = 6$, * $p < 0.05$ vs. control group).

smooth muscle cells, endothelium-removed aortic strips were incubated with a Ca²⁺-free nutrient solution and contracted by phenylephrine, so as to promote Ca²⁺ release from SR into the cytoplasm. The results showed that pre-treatment with schisandrol A (10⁻³ M) remarkably affected phenylephrine-induced vasoconstriction (Figure 5),

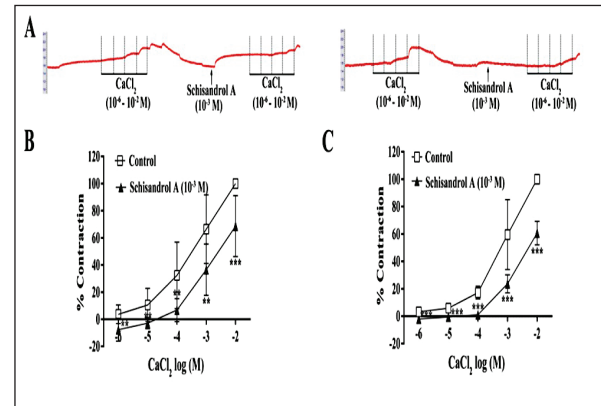


Figure 6. Effect of Extracellular Ca²⁺ Influx on the Schisandrol A-induced Vasorelaxation. (A) Typical Tracings of the Responses About Schisandrol A on the Strips Stimulated by CaCl₂ (10⁻⁶–10⁻² M) Under a Ca²⁺-free. Effects of Schisandrol A at 10⁻³ M on Aortic Strips Incubated in a Ca²⁺-free Solution and Contracted with PE 10⁻⁶ M (B) or KCl 60 mM (C) Before Addition of Cumulative Concentration of CaCl₂ (10⁻⁶–10⁻² M).

Note: The data are shown as the means $\pm$ SD ($n = 6$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle).

indicating that the endothelium-independent vasodilation of schisandrol A might be related to the inhibition of Ca²⁺ release from SR.

Effect of Extracellular Ca²⁺ Influx on the Schisandrol A-induced Vasorelaxation

To investigate whether extracellular Ca²⁺ influx was associated with schisandrol A-induced vasorelaxation, the activities of receptor-operated calcium channel (ROCC) and voltage-dependent calcium channel (VDCC) were observed. Phenylephrine can promote the influx of extracellular Ca²⁺ into the cytoplasm by activating ROCC, while a high concentration of KCl can promote Ca²⁺ influx by opening VDCC. Therefore, phenylephrine and KCl were employed to contract the endothelium-removed aortic strips in a Ca²⁺-free solution separately. It was found that schisandrol A not only weakened the contraction produced by CaCl₂ + phenylephrine but also inhibited the contraction produced by CaCl₂ + KCl (Figure 6), indicating that endothelium-independent vasodilation by schisandrol A might be partially related to double blockade of VDCC and ROCC.

Effect of Schisandrol A on eNOS Expression

To explore the effect of schisandrol A on eNOS activity, the expressions of eNOS mRNA and protein in isolated thoracic aorta were further detected. As shown in Figure 7, schisandrol A treatment upregulated expression levels of eNOS mRNA and p-eNOS after incubation for 15 min, indicating that schisandrol A might further promote NO synthesis by

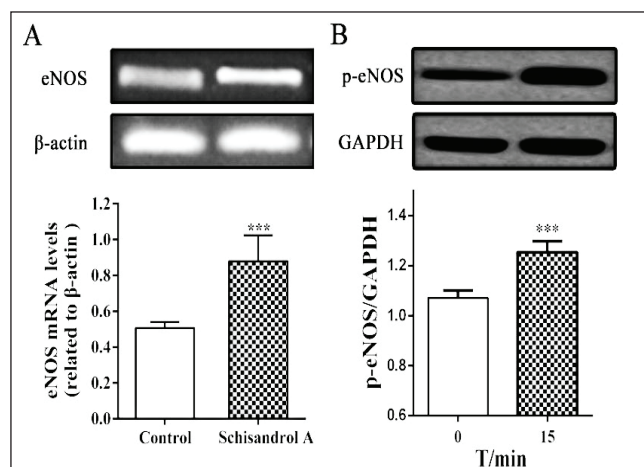


Figure 7. Effects of Schisandrol A on eNOS mRNA and Protein Expressions in the Isolated Thoracic Aorta Tissue of Rats. (A) The Level of eNOS mRNA Expression. (B) The Relative Expression of p-eNOS Protein.

Note: The data are shown as the means $\pm$ SD ($n = 3$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. vehicle).

regulating eNOS activity to induce its endothelium-dependent vasorelaxation.

Discussion

Schisandra chinensis has been applied in the treatment of a variety of diseases, and it is mainly distributed in Northeast China, Russia, Japan, and the Far East of South Korea (Panossian & Wikman, 2008; Rybníkář et al., 2019). The Treasure of Eastern Medicine, a Korean traditional medicine book, also introduced the cardiovascular effects of *S. chinensis* and clearly pointed out that *S. chinensis* could restore blood pressure and heart rhythm in patients with hypertension. Clinically, *S. chinensis* is often used in compound preparations, such as Shengmai Powder, to treat cardiovascular diseases in China (Jiang et al., 2020; Li et al., 1996). However, there are few reports on the material basis and mechanism of *S. chinensis* impacting cardiovascular function. In recent years, *S. chinensis* fruit extracts have attracted attention because of their potential roles in treating cardiovascular diseases such as myocardial infarction and hypertension (Alexander & Wang, 2012; Panossian & Wikman, 2008; Park et al., 2012). Furthermore, aqueous extract, hexane extract, gomisin A, and gomisin J from *S. chinensis* have shown relaxant effects on isolated thoracic aorta and mesenteric arteries (Park et al., 2007, 2009, 2012; Rhyu et al., 2006). With the progress of separation and analysis methods, lignans have been identified as a group of the main bioactive components in *S. chinensis* (Bártlová et al., 2002; Panossian & Wikman, 2008; Yang et al., 2022), containing a higher content of schisandrol A, schisandrol B, schisantherin A, schisandrin A, schisandrin B, schisandrin C, and so on (Chun et al., 2014). It was found in our experiments that total lignans

and several monomers could induce vasorelaxation, of which schisandrol A ($52.79 \pm 1.43\%$) and schisantherin A ($43.56 \pm 2.17\%$) had stronger vasodilation, followed by schisandrol B ($33.04 \pm 2.21\%$), while schisandrin A, schisandrin B, and schisandrin C had no significant influence on the tension of isolated rat thoracic aorta (Yang et al., 2020). These results, along with the findings of other researchers, may provide evidence that lignans should be the main active substance for *S. chinensis* to exert its vasorelaxation. In this study, it was confirmed that schisandrol A induced both endothelium-dependent and -independent vasorelaxation in isolated rat thoracic aorta pre-contracted by PE. It was also found that the relaxation in the endothelium-intact aorta was significantly greater than that in the endothelium-denuded aorta.

Endothelial-dependent vasorelaxation is primarily mediated by endothelial-derived relaxing factors (EDRFs), such as nitric oxide (NO), PGI_2 , and endothelial-derived hyperpolarizing factor (EDHF), of which NO and PGI_2 are the major vasodilators (Luksha et al., 2009). L-arginine produces NO by catalyzing eNOS, and NO activates soluble guanosine cyclase (sGC) and converts GTP into cGMP, resulting in vasodilation (Vaandrager & de Jonge, 1996). As an eNOS inhibitor, L-NAME can inhibit NO synthesis by vascular endothelial cells. In this study, the vasodilation effect induced by schisandrol A was significantly weakened in arterial strips pre-incubated with L-NAME, suggesting that NO was involved in the endothelium-dependent vasorelaxation by schisandrol A. In order to verify whether schisandrol A could promote the synthesis of NO, the isolated rat thoracic aorta was incubated with 10^{-3} M schisandrol A for 15 min, and it was found that the expressions of eNOS mRNA and protein were all raised significantly, further confirming that upregulation of eNOS activity and promotion of NO production might be involved in the endothelial-dependent vasodilation induced by schisandrol A. These results are similar to Park's research results that eNOS and NO contribute to the vasorelaxation induced by gomisin A and gomisin J (Park et al., 2007, 2012). Besides, PGI_2 in vascular endothelial cells is derived from arachidonic acid catalyzed by COX (cyclooxygenase). However, the vasodilation of schisandrol A on the thoracic aorta strips pre-incubated with a COX inhibitor indomethacin did not significantly change, indicating that PGI_2 might not be involved in this vasodilation.

Endothelial-independent vasorelaxation is primarily mediated by regulating various ion channels on the vascular smooth muscle cell membrane (VSCM), such as potassium and calcium channels. Four kinds of potassium channels, K_{IR} , K_{V} , K_{ATP} , and $\text{K}_{\text{Ca}^{2+}}$, have been found now on VSCM (Dogan et al., 2019). The opening of these potassium channels can induce cell membrane hyperpolarization, reduce calcium influx, and lead to vasorelaxation. In this study, pre-incubating with four inhibitors of potassium channels, namely, BaCl_2 (K_{IR} inhibitor), TEA (K_{V} inhibitor), 4-AP (K_{ATP} inhibitor), and glibenclamide ($\text{K}_{\text{Ca}^{2+}}$ inhibitor), did not affect the vasodilation of schisandrol

A on the endothelium-removed aortic strips, indicating that the endothelium-independent vasodilation of schisandrol A may not be related to the potassium channel opening. The calcium channel is the main ion channel to maintain vascular tension, mainly including ROCC and VDCC (Sandoo et al., 2010). Phenylephrine can activate ROCC and then regulate the inflow of extracellular calcium, resulting in vasoconstriction (Chen et al., 2009). Our study found that the incubation of schisandrol A with phenylephrine could significantly reduce the vasoconstrictive effect of CaCl_2 , suggesting that ROCC may be involved in schisandrol A-induced vasorelaxation. Phenylephrine activates the specific receptor of the sarcoplasmic reticulum to give rise to Ca^{2+} release, which increases the concentration of intracellular calcium and then causes a transient contractile response (Senejoux et al., 2013). Pre-incubation with schisandrol A significantly inhibited the transient vasoconstriction of phenylephrine on the strips, suggesting that schisandrol A-induced vasodilation may be related to its inhibiting the calcium release from the sarcoplasmic reticulum. High potassium promotes VDCC opening and depolarizes cell membranes (Hu et al., 2018). These results demonstrated that schisandrol A could significantly weaken the vasoconstriction caused by CaCl_2 under the condition of high potassium, indicating that the vasodilation induced by schisandrol A may be related to its inhibiting VDCC opening.

Our previously published data showed that schisantherin A, another main lignan ingredient of *S. chinensis*, could cause an endothelium-dependent relaxation via NO and PGI_2 pathways, as well as an endothelium-independent relaxation by the blockade of VDCC (Yang et al., 2020). The results regarding the mechanism are different from the vasodilation mechanism of schisandrol A. Further studies are needed to elucidate the structure–effect relationship regarding the different monomers of *S. chinensis* and the pathways involved in their activating eNOS or blocking calcium channels.

Summary

In sum, this study demonstrates that schisandrol A can induce both the endothelium-dependent and -independent vasorelaxation on isolated rat thoracic aorta for the first time, and the synthesis of NO and the activity of calcium channel may contribute to the mechanism. The results may provide experimental evidence for the research and development of *S. chinensis*. However, the specific mechanism of this effect is still unclear, and further research is necessary to evaluate the effect of schisandrol A on vasorelaxation.

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Abbreviations

S. chinensis: *Schisandra chinensis*; PE: Phenylephrine; L-NAME: N ω -nitro-L-arginine methyl ester; 4-AP: 4-aminopyridine; BaCl_2 : Barium chloride; TEA: Tetraethylamine; eNOS: Endothelial nitric oxide synthetase; ROCC: Receptor-operated calcium channel; VDCC: Voltage-dependent calcium channel; EDRFs: Endothelial-derived relaxing factors; NO: Nitric oxide; EDHF: Endothelial-derived hyperpolarizing factor; sGC: Soluble guanosine cyclase; COX: Cyclooxygenase; VSCM: Vascular smooth muscle cell membrane.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

All animal experiments were approved by the Experimental Animal Ethics Committee of Beihua University (Jilin, China). All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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