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Original Article

Efficacy of the Optimal Dosage of Lisinopril in Inhibiting Myofibroblast Differentiation for Attenuating Rheumatic Heart Disease Progression: An in Vitro Study

Achmad Lefi, MD*, Denisa Nugrahita Asmarani, MD, Budi Baktijasa Dharmadjati, MD, Denny Suwanto, MD, Mahendra Eko Saputra, MD, Vemaniarti Lian Pravitasari, MD, Faizal Ablansah Anandita, MD

Department of Cardiology and Vascular Medicine, Faculty of Medicine Universitas Airlangga-Dr. Soetomo General Hospital, Surabaya, Indonesia.

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Abstract

Background: *Rheumatic heart disease (RHD) is exacerbated by chronic inflammation that stimulates the release of proinflammatory cytokines, most notably transforming growth factor-beta 1 (TGF-β1), which promotes myofibroblast differentiation. This study aims to determine the optimal dosage of Lisinopril, an angiotensin-converting enzyme inhibitor, for mitigating the fibrotic changes associated with RHD.*

Methods: *This in vitro, posttest-only control group study involved obtaining valvular interstitial cells from the heart valves of 25 male New Zealand rabbits (Oryctolagus cuniculus). Valvular interstitial cells were divided into 5 groups: a control group exposed to TGF-β1, and 4 experimental groups exposed to various Lisinopril doses (1 μM, 10 μM, and 100 μM) in addition to TGF-β1. The effect of Lisinopril on myofibroblast differentiation was assessed by measuring alpha-smooth muscle actin (αSMA) expression through immunocytochemical methods. Statistical significance was determined using an independent T-test with a P value of less than 0.050.*

Results: *Independent T-tests conducted on 25 male Oryctolagus cuniculus demonstrated significantly lower αSMA expression in the groups treated with various Lisinopril doses (1 μM, 10 μM, and 100 μM) compared with the TGF-β1-induced control group (P<0.050). The most significant reduction in αSMA expression was observed in the group treated with the highest Lisinopril dose of 100 μM.*

Conclusion: *Lisinopril demonstrates a significant ability to inhibit TGF-β1-induced myofibroblast differentiation in rabbit valve interstitial cells, with the 100 μM dose proving most effective. These results suggest that Lisinopril may have the potential to curb RHD progression, warranting further investigations in vivo.*

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Keywords: *Lisinopril; Rheumatic heart disease; Interstitial cell; Myofibroblast; Transforming growth factor beta 1*

*Corresponding Author: ***Achmad Lefi,*** *Associate Professor of Cardiology, Department of Cardiology and Vascular Medicine, Faculty of Medicine Universitas Airlangga, Dr Soetomo General Hospital, Jalan Mayjend Prof. Dr Moestopo 6-8, Surabaya 60286, Indonesia. Tel: +62 811356160. E-mail: achmad.lefi@fk.unair.ac.id.*

Introduction

Despite progress in addressing the global burden of rheumatic heart disease (RHD), the condition remains a significant challenge, primarily affecting disadvantaged populations with limited access to primary healthcare.[1] In 2015, an estimated 33 million people (0.4% of the global population) were affected by RHD, with high concentrations in sub-Saharan Africa, South Asia, and the Asia-Pacific region.[2] The World Heart Federation (WHF) has intensified its campaign, aiming for a 25.1% reduction in RHD burden, with a near-elimination goal for low- and middle-income countries by 2025. Although the diagnosis and management of advanced RHD continue to present significant challenges, addressing existing gaps in care may hold the key to achieving this goal. Focusing on disease progression mitigation could play a crucial role in this effort.[1]

Persistent damage to heart valves in RHD is attributable to severe or recurrent episodes of acute rheumatic fever. This type of fever is an autoimmune response initiated by Streptococcus pyogenes (commonly known as Group A Streptococcus, or GAS) pharyngeal infection.[3,4] In tissue injuries, such as those affecting valve tissue, inflammation leads to the release of proinflammatory cytokines, including transforming growth factor-beta 1 (TGF-β1). TGF-β1 induces the activation of the SMAD signaling pathway, mitogen-activated protein kinase (MAPK), and extracellular signal-regulated kinase 1/2 (ERK1/2), ultimately causing myofibroblast differentiation and extracellular matrix synthesis. This myofibroblast differentiation plays a pivotal role in the fibrosis, calcification, and alterations of the extracellular matrix structure observed in heart valves during RHD progression.[5]

The inflammatory cascade in RHD presents an opportunity for employing antifibrotic agents to potentially disrupt the progression from acute rheumatic fever to valve pathology.[6] Angiotensin-converting enzyme (ACE), a widely expressed glycoprotein,[7] plays a critical role in the renin-angiotensin system (RAS), converting angiotensin I into angiotensin II, which primarily acts as a vasoconstrictor. Recent discoveries have highlighted the influence of angiotensin II on cell proliferation[8] and inflammatory responses, including tissue fibrosis, via the activation of SMAD and TAK1 pathways by TGF-β1.[9] Inhibition of the ACE pathway through the use of ACE inhibitors at suitable doses has been shown to suppress the TGF-β1/SMAD2/3 and TGF-β1/TAK1 activation pathways, ultimately inhibiting fibrosis or tissue scarring formation.[9]

Lisinopril is a non-sulfhydryl ACE inhibitor that is absorbed in its active form and does not undergo hepatic metabolism.[10] A study on TGF-β-induced rat fibroblast cells demonstrated significant inhibition of fibroblast proliferation into fibrotic tissue upon the administration of Lisinopril at a dose of 10 μM.[9]

A study on cardiomyocytes by Scisciola et al[11] demonstrated antioxidative, anti-inflammatory, antifibrotic, and anti-apoptotic effects of Lisinopril, which increased with doses up to 100 μM through the activation of Sirtuin 1 and Sirtuin 6 pathways. Nonetheless, no study has established an optimal dose or compared the effects of various Lisinopril doses. Accordingly, the present study aimed to evaluate the efficacy of Lisinopril at different doses in inhibiting valvular interstitial cell (VIC) differentiation into myofibroblasts in an in vitro model of RHD.

Methods

This experimental in vitro study with a posttest-only design was conducted at the Institute for Tropical Diseases, Airlangga University, Surabaya. VICs from New Zealand rabbits (*Oryctolagus cuniculus*) were used as the experimental units. The rabbits were identified with positive vimentin markers and negative CD31 markers. A total of 25 male New Zealand rabbits weighing 2.5 to 3 kg each were utilized for the research. After a 7-day acclimation period, the rabbits were euthanized using a lethal intraperitoneal dose of 125 mg/kg of phenytoin and 5 mg/kg of midazolam.[12]

The study received approval from the local ethics committee (227/EC/KEPK/FKUA/2023). Key materials utilized in this study included the following: alpha-smooth muscle actin (αSMA) monoclonal antibody (Invitrogen MA5-11547), recombinant human TGF-β1 (Biolegend 781804), and Lisinopril (GLPBIO GC16307).

VIC Collection and Preparation: Aortic valve leaflets were dissected using Vannas-type capsulotomy micro-scissors (3 mm blades), identified by a U-shaped base at the aorta. To release valvular endothelial cells (VECs), valves were treated with 100 μL of collagenase II (425 U/mL) for 5 minutes at 37 °C. Afterward, the valves were further digested for 2 hours with 100 μL of collagenase II (425 U/mL) to extract VICs. VICs were obtained by pipetting the solution up and down using a 200 μL pipette tip. Collagenase II was diluted in 19 mL of culture media, and the VICs, along with any remaining valve debris, were separated through centrifugation at 670 × g for 5 minutes. After discarding the supernatant, the VICs were transferred to culture plates or tubes. The VICs were incubated in culture media at 37 °C with 5.0% CO2 for 5 to 7 days until the desired confluence was achieved. The medium was changed after 72 hours. After resuspending the pellets in alpha minimal essential medium (α-MEM) with 10.0% fetal bovine serum (FBS), 80 cm^2 culture tubes were filled with a 1.0% penicillin/streptomycin solution. The cell cultures were then pre-incubated for 4 hours at 37 °C in 95.0% humidity (humidified air with 5.0% CO_2). Non-adherent cells were aspirated and replaced with fresh α-MEM, which contained a 1.0% penicillin/streptomycin solution and 10.0% FBS. VIC cells doubled in proliferation every 30 hours, achieving

80.0% confluence within 5 days. Phase-contrast microscopy was employed on days 1, 3, 5, 7, and 9 to examine the cell cultures. The medium was changed every 2 to 3 days, and cells were grown to 80.0% confluence before being passaged using trypsin-EDTA. On the subsequent day, VIC cells were subcultured (1:3 dilution) to passage 1. Cells were then split every 4 hours and cultured until passage 3. Passage 1, 2, and 3 cells were plated at a density of 0.3×105 cells/cm² and cultured for 24 hours, followed by a 24-hour serum starvation process before treatment. Phenotypic markers were monitored during characterization, with vimentin serving as a positive marker and CD31 as a negative marker for VICs.

TGF- β1 Exposure and Lisinopril Dosage: After the cell culture reached 80.0% to 90.0% confluence in α-MEM with 10.0% FBS in multi-well plates, passage 3 VIC cells were seeded at a density of 50,000 cells/cm³. The cells were cultured in α-MEM with 1.0% FBS (serum deprivation for 24 hours).[13,14]

The VIC cells were divided into 5 groups:

- Group P1: No treatment (positive control).
- Group P2: Pretreated with 1 μM of Lisinopril for 48 hours, followed by incubation with 5 ng/mL of TGF-β1 for 72 hours.
- Group P3: Pretreated with 10 μM of Lisinopril for 48 hours, followed by incubation with 5 ng/mL of TGF-β1 for 72 hours.
- Group P4: Pretreated with 100 μM of Lisinopril for 48 hours, followed by incubation with 5 ng/mL of TGF-β1 for 72 hours.
- Group P5: Treated only with 5 ng/mL of TGF-β1 for 72 hours (negative control).

At the end of the experimental protocol, the cell layer was washed twice with PBS solution and permeabilized with absolute methanol for 7 minutes.[15]

Measurement of Myofibroblast Differentiation (αSMA Expression): Myofibroblast differentiation, marked by the expression of the contractile protein αSMA, was assessed via an immunocytochemical staining method. After exposure to the intervention agent, cells were fixed with 2.0% paraformaldehyde/PBS and 1.0% Triton X-100/PBS for 30 minutes. Next, αSMA was identified using an avidin biotinylated enzyme. Cells were initially blocked with 2.0% BSA/PBS, followed by quenching of endogenous peroxide with 3.0% H_2O_2. Subsequently, the cells were incubated for 60 minutes with a rabbit monoclonal antibody against αSMA (Sigma), and then for 20 minutes with additional Avidin-conjugated peroxidase (Sigma) and a biotinylated secondary antibody (goat anti-mouse IgG; Sigma). Following the antibody incubation period, the cells were washed 3 times for 5 minutes each with PBS. The 3-amino-9-ethylcarbazole substrate (Zymed Laboratories, South San Francisco, CA) was used to visualize the staining. Afterward, the cells were counterstained with hematoxylin to reveal the nuclei and count the number of cells (>500/slide). Cells exhibiting a positive reaction for αSMA staining appeared as rose-red to brownish-red colored filaments in the cytoplasm and were expressed as a percentage of the total cell population.

Results were expressed as mean ± standard deviation (SD) for variables with a normal distribution. All experimental results were analyzed using an independent T-test. Statistical tests were performed using SPSS Statistics, version 26. Statistical significance was set at a P value of less than 0.050.

Results

Isolated VIC cells were identified using a light microscope. The cell culture isolation process yielded cells with a spindle shape and regular cell membranes. To confirm successful VIC isolation, immunocytochemical analysis was performed using vimentin to differentiate between VECs and VICs. Under a phase-contrast microscope, VIC cell morphology appeared spindle-shaped, lacking the cobblestone pattern characteristic of VECs. Vimentin staining, observed via fluorescence microscopy, served as a positive marker for VICs (Figure 1). A total of 145 VIC cell samples were collected and divided into 5 treatment groups. The study included 2 control groups: a positive control group consisting of untreated samples without TGF-β1 administration, and a negative control group comprising samples treated with TGF-β1 only, without Lisinopril administration (Table 1). Our analysis revealed a significant increase in αSMA expression in both groups, with Group P5 exhibiting a mean difference of 8.70±0.40 (P<0.050). The finding demonstrated a significant differentiation of VICs into myofibroblasts upon the administration of TGF-β1. Figures 2 to 5 display αSMA expression in both the control and treatment groups. The intensity of luminescence was observable in each group, with brighter luminescence indicating higher αSMA expression. The independent T-test revealed significantly lower αSMA expression in groups treated with various doses of Lisinopril than in the group treated with TGF-β1 alone (Lisinopril doses: 1 μM, 10 μM, and 100 μM; P<0.050). The group administered Lisinopril at 100 μM exhibited the lowest and most significant reduction in αSMA expression. As shown in Table 1, the mean differences in other groups were significantly higher than in the group treated with 100 μM of Lisinopril (P<0.050). Nevertheless, αSMA expression in the group given 1 μM of Lisinopril was not significantly different from the group administered Lisinopril at 10 μM (P>0.050).

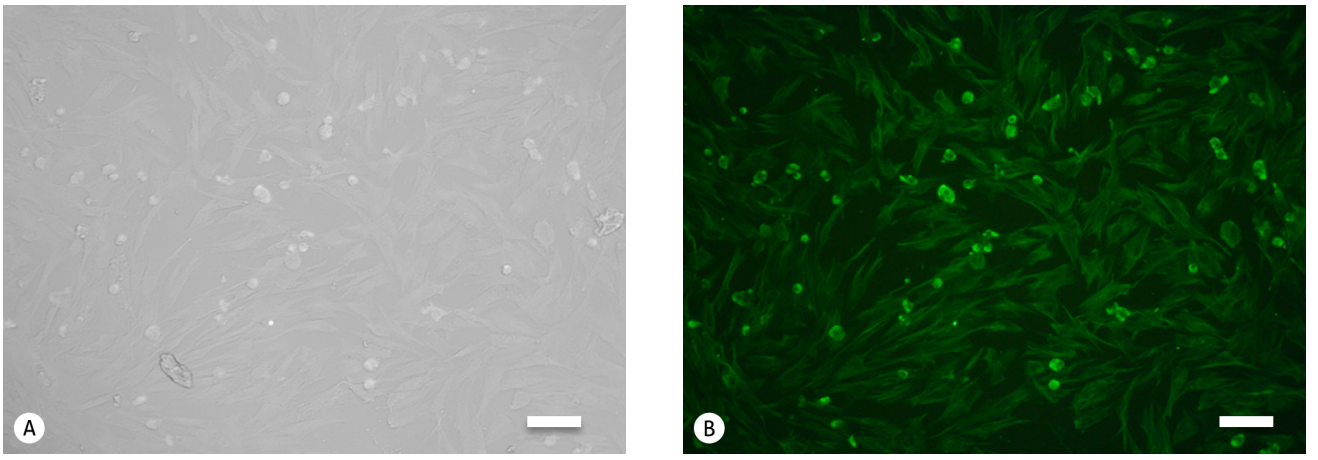

Figure 1. The images showcase vimentin expression following valvular interstitial cell isolation. The left panel (A) shows that the valvular interstitial cell morphology is spindle-shaped, while the cobblestone morphology characteristic of valvular endothelial cells is not observed. The right panel (B) displays the luminescence of vimentin under a fluorescent microscope, indicating a positive marker for vimentin. Scale bar: 100 µm

Table 1. Levels of αSMA expression with and without TGF-β1 exposure and Lisinopril treatment

Reference Group	Mean (±SD) of the Refer-ence Group	Comparison Group	Mean (±SD) of the Comparison Group	Mean Difference (±SD)	P (<0.050)
Control	14.05±1.19	TGF-β1 + Lisinopril 1 µM	19.61±3.49	-5.56±0.70	0.000
		TGF-β1 + Lisinopril 10 µM	20.80±2.34	-6.75±0.49	0.000
		TGF-β1 + Lisinopril 100 µM	13.09±2.18	+0.96±0.46*	0.041
		TGF-β1	22.75±1.63	-8.70±0.40	0.000
TGF-β1 + Lis-inopril 1 µM	19.61±3.49	Control	14.05±1.19	+5.56±0.70*	0.000
		TGF-β1 + Lisinopril 10 µM	20.80±2.34	-1.19±0.77	0.128
		TGF-β1 + Lisinopril 100 µM	13.09±2.18	+6.52±0.75*	0.000
		TGF-β1	22.75±1.63	-3.14±0.71	0.000
TGF-β1 + Lis-inopril 10 µM	20.80±2.34	Control	14.05±1.19	+6.75±0.49*	0.000
		TGF-β1 + Lisinopril 1 µM	19.61±3.49	+1.19±0.77*	0.128
		TGF-β1 + Lisinopril 100 µM	13.09±2.18	+7.71±0.55*	0.000
		TGF-β1	22.75±1.63	-1.96±0.51*	0.000
TGF-β1 + Lis-inopril 100 µM	13.09±2.18	Control	14.05±1.19	-0.96±0.46	0.041
		TGF-β1 + Lisinopril 1 µM	19.61±3.49	-6.52±0.75	0.000
		TGF-β1 + Lisinopril 10 µM	20.80±2.34	-7.71±0.55	0.000
		TGF-β1	22.76±1.63	-9.66±0.47	0.000
TGF-β1	22.75±1.63	Control	14.05±1.19	+8.70±0.40*	0.000
		TGF-β1 + Lisinopril 1 µM	19.61±3.49	+3.14±0.71*	0.000
		TGF-β1 + Lisinopril 10 µM	20.80±2.34	+1.96±0.51*	0.000
		TGF-β1 + Lisinopril 100 µM	13.09±2.18	+9.66±0.47*	0.000

*The mean of the comparison group is lower than that of the reference group.
αSMA, Alpha-smooth muscle actin; TGF-β1, Transforming growth factor-beta 1

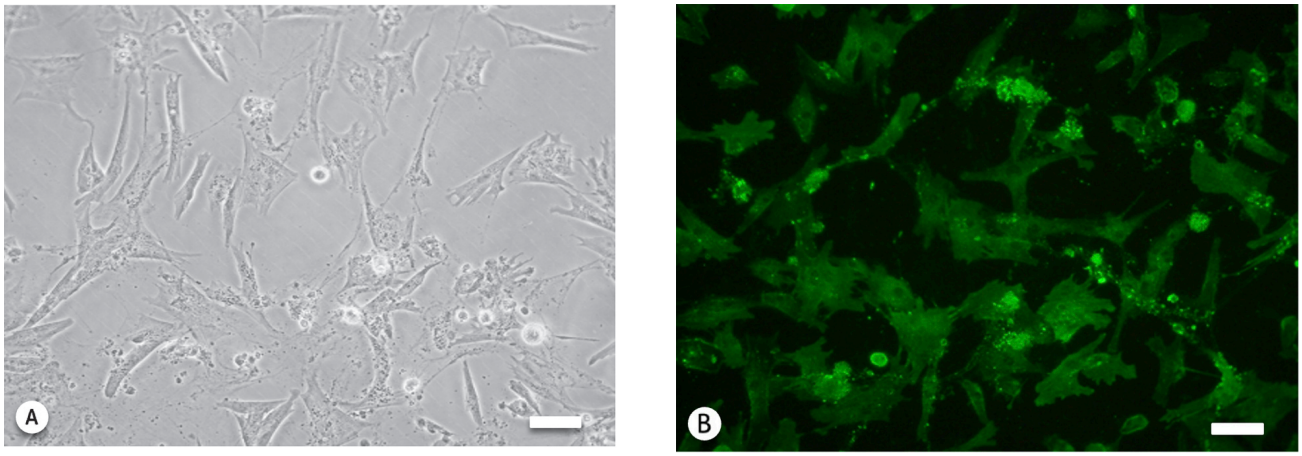

Figure 2. The images showcase the expression of αSMA in the control group. Figure A shows the VIC morphology, and Figure B demonstrates the luminescence of vimentin. White bar: 100 µm
αSMA, Alpha-smooth muscle actin; VIC, Valvular interstitial cell

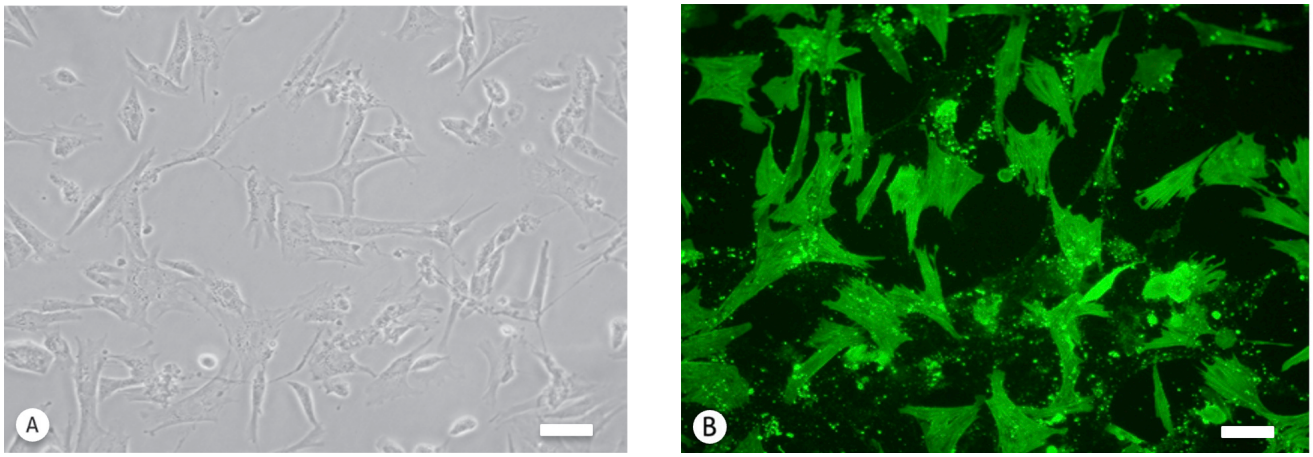

Figure 3. The images illustrate the expression of αSMA in the group given TGF-β1 and 1 µM of Lisinopril. Figure A shows the VIC morphology, and Figure B demonstrates the luminescence of vimentin. White bar: 100 µm
αSMA, Alpha-smooth muscle actin; TGF-β1, Transforming growth factor-beta 1; VIC, Valvular interstitial cell

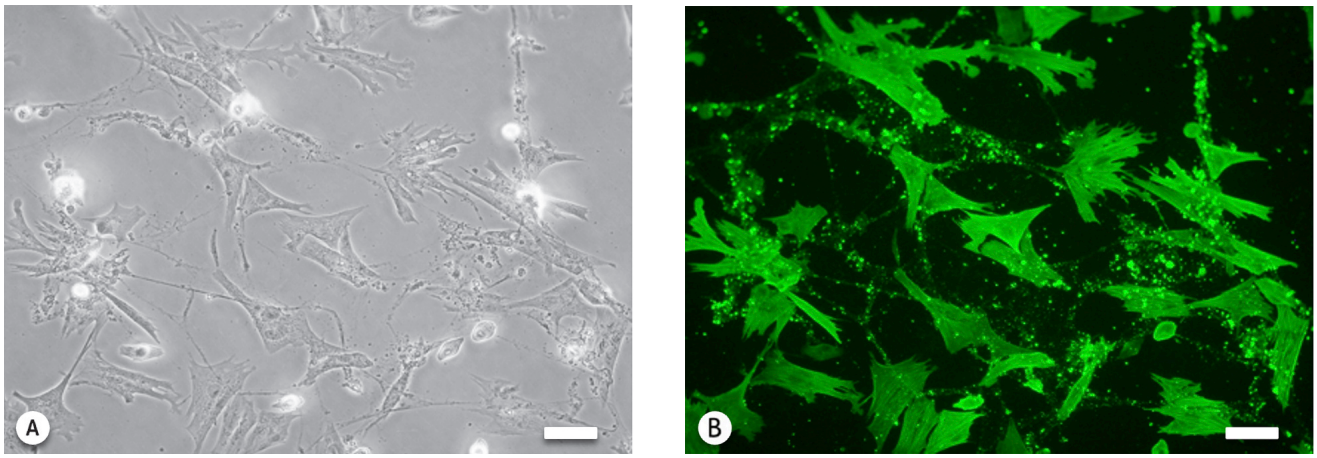

Figure 4. The images present the expression of αSMA in the group given TGF-β1 and 1 µM of Lisinopril. Figure A demonstrates the VIC morphology, and Figure B shows the luminescence of vimentin. White bar: 100 µm
αSMA, Alpha-smooth muscle actin; TGF-β1, Transforming growth factor-beta 1; VIC, Valvular interstitial cell

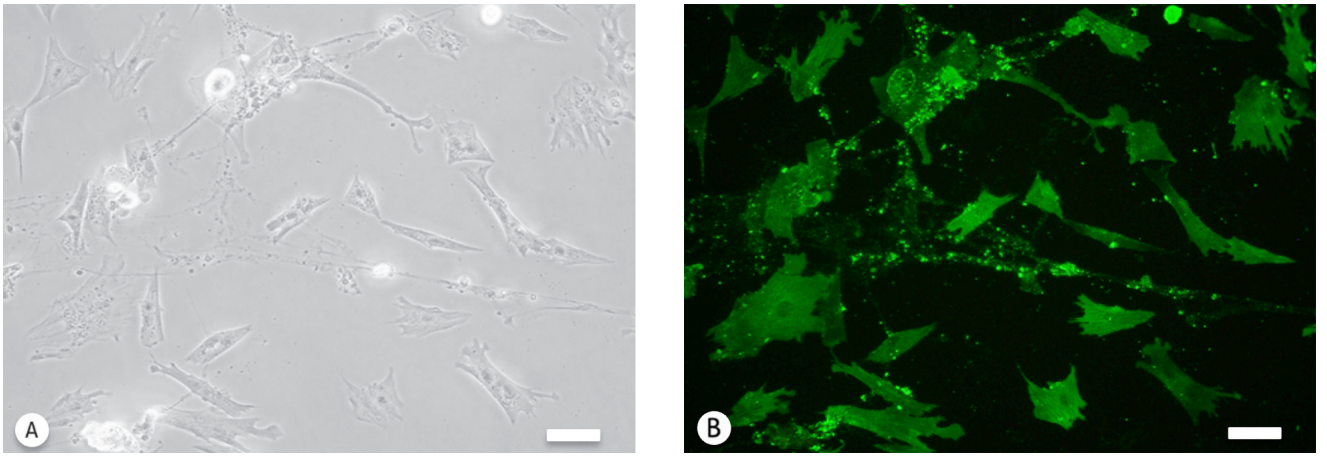

Figure 5. The images illustrate the expression of αSMA in the group given TGF-β1 and 100 μM of Lisinopril. Figure A shows the VIC morphology, and Figure B showcases the luminescence of vimentin. White bar: 100 μm
αSMA, Alpha-smooth muscle actin; TGF-β1, Transforming growth factor-beta 1; VIC, Valvular interstitial cell

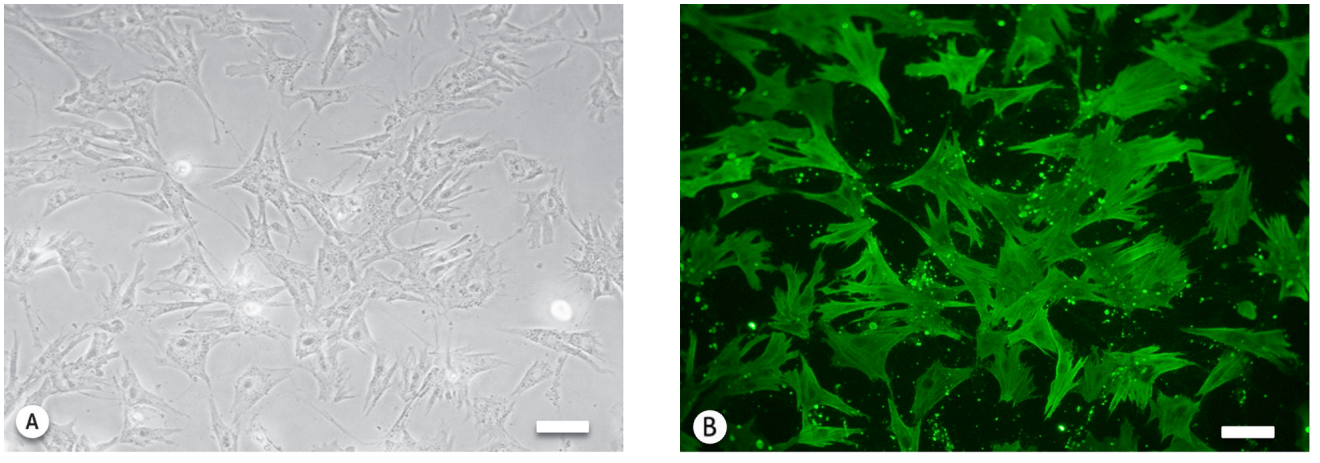

Figure 6. The images show the expression of αSMA in the group given only TGF-β. Figure A shows the VIC morphology, and Figure B demonstrates the luminescence of vimentin. White bar: 100 μm
αSMA, Alpha-smooth muscle actin; TGF-β1, Transforming growth factor-beta 1; VIC, Valvular interstitial cell

Discussion

Collectively, our results suggest that αSMA expression significantly decreased in the group treated with Lisinopril at a dose of 100 μM compared with the positive control group not treated with TGF-β1 (as indicated by luminescence intensity). This finding is consistent with a study by Fang et al,[16] which reported a decrease in collagen content in fibroblast tissue derived from rat skin without inflammatory inducers. Their study showed stronger inhibition of collagen production with Lisinopril at 100 μM compared with Lisinopril doses of 0 μM, 1 μM, and 10 μM.

The observed decrease in α-SMA expression with the administration of Lisinopril at 1 μM is consistent with a study by Sciciola et al.[11] Their research demonstrated a reduction in TGF-β1 production based on Western blot analysis in cultured human cardiomyocytes treated with TGF-β1 and Lisinopril at 1 μM and 10 μM. However, this reduction was not statistically significant compared with the control group. Two additional studies investigating fibroblast cells derived from rat skin and induced fibrosis with TGF-β1 also reported decreased TGF-β1 production with Lisinopril at 1 μM, although the difference was not significant compared with the control group.[9, 16] All 3 studies directly measured TGF-β1 levels using Western blotting and flow cytometry, revealing a drop in TGF-β1 levels upon treatment with the ACE inhibitor Lisinopril.

Another noteworthy finding from this research is the potential local production of ACE and other RAS components in tissues, particularly in cardiac fibroblasts, in response to TGF-β1-induced inflammation and myofibroblast formation. This finding was supported by the high levels of αSMA expression observed in response to TGF-β1 alone (the negative control group). In a study by Fang et al,[16] in vitro fibrosis was induced using TGF-β1 in fibroblast cells isolated from rat skin. The authors also reported an elevation

in total collagen content following TGF-β1 administration, indicating fibroblast differentiation into fibrotic tissue.

Previous studies on the isolation of heart VICs have reported a significant increase in α-SMA expression in vitro upon TGF-β1 induction compared with non-induced cells.[17] In another study by Petrov et al,[18] fibroblast cells were isolated from healthy rat ventricles and treated with TGF-β1, resulting in increased ACE activity and ACE protein levels, along with elevated α-SMA expression. Their findings suggested that TGF-β1 played a role in inducing increased ACE activity and protein levels in ventricular myocytes. In contrast, only minimal amounts of ACE were found in healthy ventricular myocytes.

As a non-sulfhydryl ACE inhibitor, Lisinopril does not require metabolism in the liver to exert its therapeutic effects. Lisinopril is water-soluble and is primarily excreted through the kidneys, with a duration of action of up to 24 hours.[10] Numerous in vitro studies have highlighted Lisinopril's significant inhibitory effects on TGF-β1. For instance, Katwa et al[19] demonstrated that in isolated VICs, Lisinopril at 50 μM was able to completely inhibit ACE mRNA expression, ACE binding activity, and the conversion of Ang I to Ang II.

Other studies have proposed that Lisinopril can inhibit both canonical and noncanonical TGF-β1 pathways.[16] The observed decrease in αSMA expression with increasing Lisinopril doses suggests a dose-dependent inhibition of myofibroblast differentiation by Lisinopril.[11] In the current study, the lowest fibrosis marker was observed with Lisinopril at 100 μM, indicating that this may be the optimal dose for ACE inhibitors in reducing tissue fibrosis by inhibiting TGF-β1 activity in an in vitro RHD model.

The findings of this study suggest a potential role for Lisinopril in attenuating RHD progression in in vivo studies. It is plausible that higher doses of Lisinopril may offer greater protection against the effects of inducers at the in vivo research level. Alternatively, lower doses might also significantly reduce myofibroblast differentiation. Further investigation into Lisinopril dosage at the in vivo level is warranted to explore its potential application in future studies.

Conclusion

Our findings demonstrated that Lisinopril effectively inhibited TGF-β1-induced myofibroblast formation in rabbit VICs at concentrations of 1 μM, 10 μM, and 100 μM, as evidenced by αSMA expression. The lowest fibrosis marker was observed with 100 μM of Lisinopril, suggesting that this concentration may represent the optimal dose of ACE inhibitors in reducing tissue fibrosis in an in vitro RHD model. These results highlight the potential of Lisinopril as a therapeutic agent for attenuating RHD progression in future in vivo studies.

Acknowledgments

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References

1. Kumar RK, Antunes MJ, Beaton A, Mirabel M, Nkomo VT, Okello E, Regmi PR, Reményi B, Sliwa-Hähnle K, Zühlke LJ, Sable C; American Heart Association Council on Lifelong Congenital Heart Disease and Heart Health in the Young; Council on Cardiovascular and Stroke Nursing; and Council on Clinical Cardiology. Contemporary Diagnosis and Management of Rheumatic Heart Disease: Implications for Closing the Gap: A Scientific Statement From the American Heart Association. Circulation 2020;142:e337-e357.
2. Watkins DA, Johnson CO, Colquhoun SM, Karthikeyan G, Beaton A, Bukhman G, Forouzanfar MH, Longenecker CT, Mayosi BM, Mensah GA, Nascimento BR, Ribeiro ALP, Sable CA, Steer AC, Naghavi M, Mokdad AH, Murray CJL, Vos T, Carapetis JR, Roth GA. Global, Regional, and National Burden of Rheumatic Heart Disease, 1990-2015. N Engl J Med 2017;377:713-722.
3. Carapetis JR, Beaton A, Cunningham MW, Guilherme L, Karthikeyan G, Mayosi BM, Sable C, Steer A, Wilson N, Wyber R, Zühlke L. Acute rheumatic fever and rheumatic heart disease. Nat Rev Dis Primers 2016;2:15084.
4. Acute rheumatic fever and rheumatic heart disease. Nat Rev Dis Primers 2016;2:15085.
5. Kim L, Kim DK, Yang WI, Shin DH, Jung IM, Park HK, Chang BC. Overexpression of transforming growth factor-beta 1 in the valvular fibrosis of chronic rheumatic heart disease. J Korean Med Sci 2008;23:41-48.
6. Vaideeswar P, Butany J. Chapter 12 - Valvular Heart Disease. In: Maximilian Buja L, Butany J, eds. Cardiovascular Pathology. 4th ed. Academic Press; 2016. p. 485-528.
7. Hermawan HO, Ardiana M, Suryawan IGR, Harsoyo PM, Rafli M. Losartan Has a Comparable Effect to Human Recombinant ACE2 in Reducing Interleukin-6 (IL-6) Levels on Human Adipocytes Exposed to SARS-CoV-2 Spike Protein. Indones Biomed J 2023;15:311-317.
8. Oktaviono YH, Hutomo SA, Luke K. The Role of Endothelial Progenitor Cells in Coronary Artery Disease: Basic Molecular Mechanisms and Its Clinical Potentials. Indones Biomed J 2021;13:106-113.
9. Tan WQ, Fang QQ, Shen XZ, Giani JF, Zhao TV, Shi P, Zhang LY, Khan Z, Li Y, Li L, Xu JH, Bernstein EA, Bernstein KE. Angiotensin-converting enzyme inhibitor works as a scar formation inhibitor by down-regulating Smad and TGF-β-activated kinase 1 (TAK1) pathways in mice. Br J Pharmacol 2018;175:4239-4252.
10. Lionel H. Opie and Marc A. Pfeffer. Inhibitors of the Renin-Angiotensin-Aldosterone System. In: Opie LH. Gersh BJ, eds. Drugs for the Heart: Expert Consult. 8th ed. Elsevier; 2013. p. 119-168
11. Scisciola L, Fontanella RA, Surina, Garofalo G, Rizzo MR, Paolisso G, Barbieri M. Potential Role of Lisinopril in Reducing Atherosclerotic Risk: Evidence of an Antioxidant Effect in Human Cardiomyocytes Cell Line. Front Pharmacol 2022;13:868365.
12. EFSA Panel on Animal Health and Welfare (AHAW); Saxmose Nielsen S, Alvarez J, Bicout DJ, Calistri P, Depner K, Drewe JA, Garin-Bastuji B, Gonzales Rojas JL, Gortázar Schmidt C, Michel V, Miranda Chueca MÁ, Roberts HC, Sihvonen LH, Stahl K, Velarde Calvo A, Viltrop A, Winckler C, Candiani D, Fabris C, Mosbach-Schulz O, Van der Stede Y, Spoolder H. Stunning methods and slaughter of rabbits for human consumption. EFSA J 2020;18:e05927.

13. Monzack EL, Gu X, Masters KS. Efficacy of simvastatin treatment of valvular interstitial cells varies with the extracellular environment. Arterioscler Thromb Vasc Biol 2009;29:246-253.
14. Zhang Y, Lu Y, Ong'achwa MJ, Ge L, Qian Y, Chen L, Hu X, Li F, Wei H, Zhang C, Li C, Wang Z. Resveratrol Inhibits the TGF-β1-Induced Proliferation of Cardiac Fibroblasts and Collagen Secretion by Downregulating miR-17 in Rat. Biomed Res Int 2018;2018:8730593.
15. Eghbali M, Tomek R, Woods C, Bhambi B. Cardiac fibroblasts are predisposed to convert into myocyte phenotype: specific effect of transforming growth factor beta. Proc Natl Acad Sci U S A 1991;88:795-799.
16. Fang QQ, Wang XF, Zhao WY, Ding SL, Shi BH, Xia Y, Yang H, Wu LH, Li CY, Tan WQ. Angiotensin-converting enzyme inhibitor reduces scar formation by inhibiting both canonical and noncanonical TGF-β1 pathways. Sci Rep 2018;8:3332.
17. Rutkovskiy A, Malashicheva A, Sullivan G, Bogdanova M, Kostareva A, Stensløkken KO, Fiane A, Vaage J. Valve Interstitial Cells: The Key to Understanding the Pathophysiology of Heart Valve Calcification. J Am Heart Assoc 2017;6:e006339.
18. Lijnen PJ, Petrov VV, Fagard RH. Induction of cardiac fibrosis by transforming growth factor-beta(1). Mol Genet Metab 2000;71:418-435.
19. Katwa LC, Campbell SE, Tyagi SC, Lee SJ, Cicila GT, Weber KT. Cultured myofibroblasts generate angiotensin peptides de novo. J Mol Cell Cardiol 1997;29:1375-1386.