

RESEARCH ARTICLE

Protection of Ang1-7 Through MKK/P38MAPKs Inflammatory Signal Pathway on TNF- α Stimulated Mouse HL-1 Cells

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Citation: Xuewen Wang, Yuan Cao, Pradeep Depark, Deepark Sharma, Guangping Li, et al. (2022) Protection of Ang1-7 Through MKK/P38MAPKs Inflammatory Signal Pathway on TNF- α Stimulated Mouse HL-1 Cells. J Cardiac Disord Ther 3: 101

Abstract

Background: To explore the effect of Ang1-7 through MKK/P38MAPKs inflammatory signaling pathway on TNF- α -stimulated mouse HL-1 cells.

Methods: Using TNF- α (100 μ g/ml) to establish an inflammatory atrial fibrillation model in HL-1 cell, which derived from mouse atrial myocyte. treated HL-1 cells with different concentrations of Ang 1-7 (0.1, 1 and 10 mmol/L) and divided into 5 groups, namely A group(control group), B group (TNF), C group (TNF + Ang 1-7 0.1 mmol/L), D group (TNF + Ang 1-7 1 mmol/L) and E group (TNF + Ang 1-7 10 mmol/L). Firstly, different concentrations of Ang 1-7 (0.1 mmol/L, 1 mmol/L and 10 mmol/L) were used to stimulate for half an hour, and then TNF- α (100 μ g/ml) was added to stimulate for four hours. Both the cells and supernatant were collected. Cells were collected for Western Blotting to detect the protein expression of MKK3, MKK4, MKK6, PMMK4 and PP38. The supernatant was subjected to flow cytometry for detecting multi-inflammatory factors.

Results: Compared with the A group, the protein expression of MKK3, MKK4, MKK6, PMMK4 and PP38 was statistically significant increased after stimulation with inflammatory factors (TNF- α) ($P < 0.05$). After intervention with Ang 1-7, the protein expression of MKK3, MKK4, MKK6, PMMK4 and PP38 was statistically significant lower than that of B group ($P < 0.05$). There is no significant difference of the protein expression of P38 after stimulation with inflammatory factor (TNF- α). Compared with the A group, there was no significant difference in the protein expression of MAS after the stimulation of inflammatory factor (TNF- α). After the intervention of Ang 1-7, the protein expression of MAS was higher than that of the A group and B group, but there was no significant difference ($P > 0.05$). The expression of MAS protein had an increasing trend, but there was no significant difference ($P > 0.05$). TGF- β , TNF- α was significantly increased after stimulating factor (TNF- α) was given, but was decreased after the intervention of Ang 1-7, both there were statistically significant ($P < 0.05$). IL-6 also had the same trend, but there was no significant difference.

Conclusion: Ang1-7 through MKK/P38MAPKs inflammatory signal pathway protected on TNF- α stimulated mouse HL-1 cells

Keywords: Ang1-7; MKK/P38MAPKs; Inflammatory Signal Pathway; HL-1 cells

Introduction

Atrial fibrillation is the most common arrhythmia; its molecular mechanism is unclear. So far, the pathophysiology mechanisms of atrial fibrillation are not fully understood, the targeted therapy for atrial fibrillation fails to meet clinical needs. Many researches have clarified that atrial remodeling is a prerequisite for atrial fibrillation [1]. Other researches show that atrial fibrosis is not only related to the stimulation of cardiomyocytes and fibroblasts, but also related to the activation state of white blood cells. With the release of reactive oxygen species, cytokines and growth factors, white blood cells are recruited and subsequent increased matrix deposition, leading to unfavorable atrial remodeling [2,3]. These inflammatory pathways are prerequisites for atrial fibrillation. The angiotensin-aldosterone-system is an important hormone system in the occurrence and development of atrial fibrillation. Therefore, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers are becoming new drugs to prevent atrial fibrillation [4]. Ang1-7 is produced by angiotensin-converting enzyme 2 (ACE2) catalyzed angiotensin I (Ang I) or angiotensin II (Ang II). The intracellular signal mechanism transmitted by MAS is still unclear. such as Akt phosphorylation, protein kinase C activation and mitogen-activated protein kinase (MAP) inhibition seem not be involved in this signal transduction pathway [5]. Wang et al. also confirm that Ang1-7 attenuates the expression of heat shock protein (HSP27), thereby inhibiting the occurrence of atrial fibrillation [6]. the Ang1-7/MAS conduction pathway should be further study and makes it an important target for the treatment of Atrial fibrillation.

Materials and Methods

Cell Culture and Groups Divided

All cell experiments required ultraviolet rays to irradiate the operating table and the items needed for the experiment for 20 - 30 minutes before subsequent experiments carried out. HL-1 atrial myocytes were cultured in DMEM high glucose medium containing 10% fetal bovine serum or DMEM high glucose medium with penicillin/streptomycin (fetal bovine serum: penicillin/streptomycin: DMEM high glucose medium 10: 1.1: 100), in 5% CO₂ incubator at 37°C. Usually we used 12-well plates for Western Blotting and flow cytometry for detecting multi-inflammatory factors. Marked the blank control group as A group and A' group, experimental group TNF- α (TNF- α (100 μ g/ml)) as B group, B' group, TNF- α + Ang 1-7 (0.1 mm) as C group, C' Group, TNF- α + Ang 1-7(1 mm) as D group, D' group, TNF- α + Ang 1-7 (10 mm) as E group, E' group. Group A and Group A' were repeated controls for each other. The HL-1 cells were given different concentrations of Ang 1-7 (0.1, 1 and 10 mmol/L) to stimulate for half an hour, and added TNF- α (100 μ g/ml) to stimulate for 4 h, in 5% CO₂ incubator at 37°C. After time was up, the cells and supernatant were collected, the cells were collected for Western Blotting, the supernatant was subjected to flow cytometry for detecting multi-inflammatory factors.

Western Blotting

Different treatments of HL-1 cells were used to extract protein from cell lysates using RAPI. Loaded 10 μ l protein samples and subjected to 10% SDS-polyacrylamide gel electrophoresis (140 MV) to separate proteins of different sizes and molecular weights. Transferred the protein to PVDF membrane, 200 MA, 1.5 h. PVDF membrane were blocked with Tris-buffered saline containing 10% nonfat milk at room temperature for 1 h, then incubated with the primary antibodies diluted in TBS at room temperature overnight. Primary antibodies used in this study were as followed: anti-P38MAPK antibody (#8690s, 1: 1000), anti-Phospho-p38mapk (Thr180/Tyr182) antibody (#4511, 1:1000), anti- β -actin antibody (1: 1000), anti-mkk3 antibody (1: 1000), anti-phospho-SEK1/mkk4 (C36C11, 1: 1000), Phospho-MKK4c36c11 (1: 1000), MKK6 (Cell signaling technology, 1: 1000), anti-MASc-390453 (1: 500). Membranes were incubated with the secondary antibody at room temperature for 1 h and visualized using the chemiluminescence reagent ECL advance. TanonGis system was used to perform band gray scale analysis to calculate the net optical density, and the ratio of the net optical density of the target protein to the net optical density of β -actin represented the relative level of the target protein.

Flow cytometry

We chose 10 supernatant EP tubes (respectively con, TNF- α , TNF- α + Ang 1-7 (0.1 mm), TNF- α + Ang 1-7 (1 mm), TNF- α + Ang 1-7 (10 mm), repeated control for each group A and A'). Added beads mouse 10 μ l + Mouse Macrophage/Microglla Panel Detection Antibodies 10 μ l + legend plex Assay Buffer 20 μ l to each EP tube and mixed. Then added 10 μ l of supernatant to each EP tube and mixed, avoided light and shaken on a vortex shaker at a speed of 3 for 1.5 h. When the time was up, PE 10 μ l + legend plex Assay Buffer 10 μ l were added to each EP tube, and continued to avoid light and shaken on the vortex oscillator at a speed of 3 for 0.5 h. Taken a 15 ml centrifuge tube and added 14.5 ml deionized water (ddH₂O) + wash Buffer (20 \times) 750 μ l into it. After the shaking was over, added 500 μ l of the liquid in the prepared 15 ml centrifuge tube, and centrifuged of 600 rcf for 8 min at 4°C. After centrifugation, blue crystal precipitation could be seen. The upper layer of liquid was removed using a micropipette, leaving blue crystals. 200 μ l of the liquid was added in the prepared 15 ml centrifuge tube and subjected to the flow cytometry for multi-factor detection.

Statistical analysis

The results were compared with statistics using SPSS 25.0 software. Measurement data were expressed as mean \pm standard deviation ($\bar{x} \pm s$). Significance test was performed using one-way analysis of variance (ANOVA), and LSD was used for comparison between groups when the test for homogeneity of variance was equal. When the homogeneity of variance is unequal, the comparison between the groups uses the Tamhneni method. $P < 0.05$ was considered statistically significant.

Results

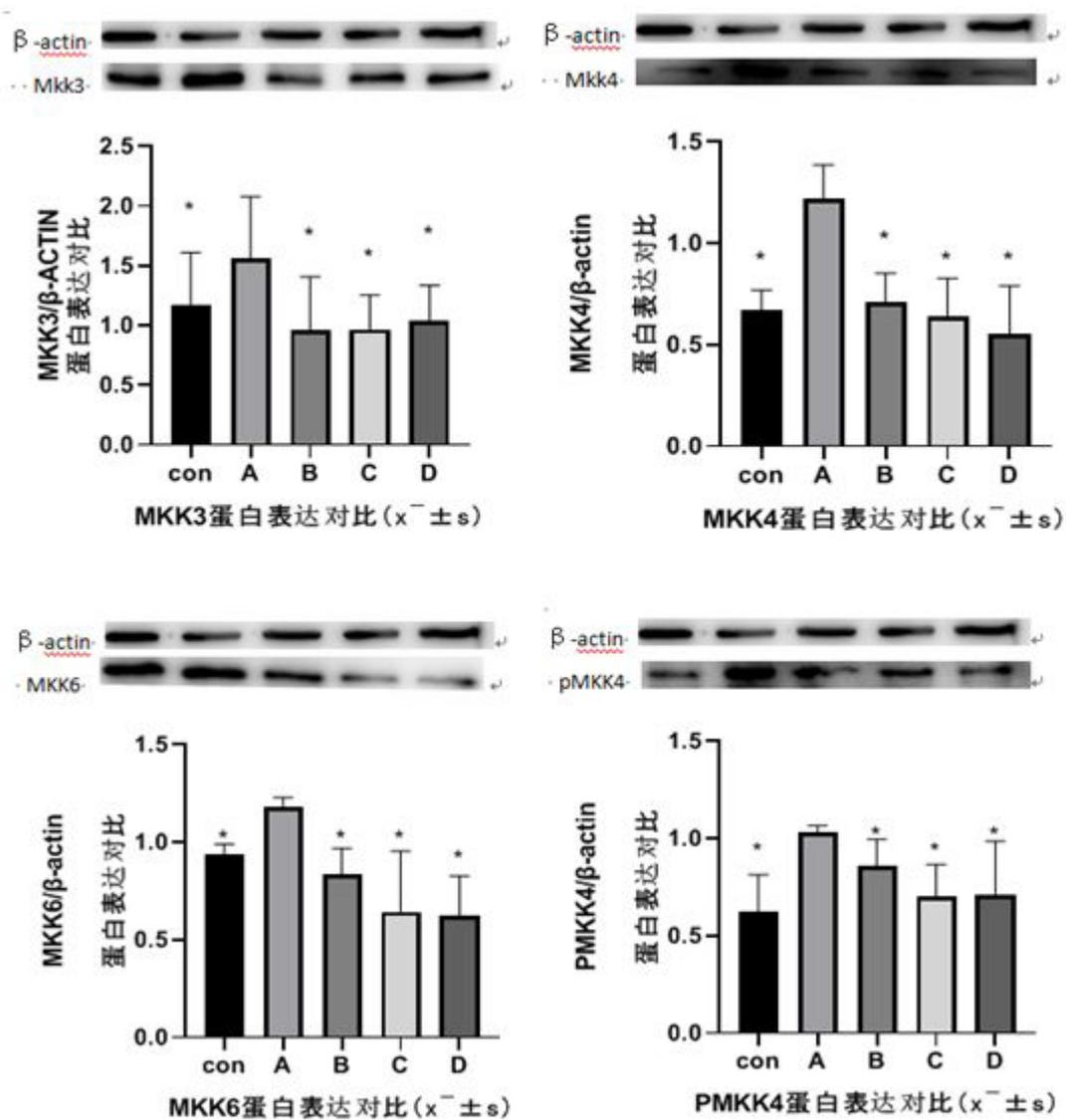
Relative protein expression of MKK3/4/6 and PMKK4

Compared with the A group, the protein expressions of MKK3, MKK4, MKK6, and PMMK4 was significantly increased after stimulation with inflammatory factors (TNF- α), which was statistically significant ($P < 0.05$). After intervention with Ang 1-7, the protein expression of MKK3, MKK4, MKK6, and PMMK4 was significantly lower than that of the stimulation group ($P < 0.05$). The protein expressions of MKK4, MKK6 and PMMK4 between groups B, C, and D, E showed an increasing trend, but there was no significant difference ($P > 0.05$). There is no significant difference in multiple comparisons of MKK3, MKK4, MKK6, PMMK4 between groups B, C, and D,E ($P > 0.05$) (Table 1 and Fig 1).

Group	n	MKK3	MKK4	MKK6	Pmkk4
A Group(Control)	6	1.06 \pm 0.217*	0.78 \pm 0.215*	0.92 \pm 0.091*	0.66 \pm 0.165*
B Group(TNF- α)	6	1.49 \pm 0.323	1.20 \pm 0.139	1.22 \pm 0.089	1.11 \pm 0.126
C Group(TNF- α + Ang 1-7 0.1mm)	6	0.92 \pm 0.262*	0.61 \pm 0.199*	0.81 \pm 0.158*	0.85 \pm 0.119*
D Group(TNF- α + Ang 1-7 1 mm)	6	0.96 \pm 0.164*	0.62 \pm 0.171*	0.66 \pm 0.246*	0.74 \pm 0.181*
E Group(TNF- α + Ang 1-7 10 mm)	6	0.99 \pm 0.170*	0.58 \pm 0.216*	0.64 \pm 0.183*	0.65 \pm 0.250*

*: Compared with TNF- α group, $P < 0.05$

Table 1: Comparison of MKK3, MKK4, MKK6, PMKK4 protein expression in HL-I cells of each group($\bar{x} \pm s$)



CON: blank control group, group A: TNF- α treated, group B: TNF- α + Ang 1-7 0.1 mm, group C: TNF- α + Ang 1-7 1 mm, group D: TNF- α + Ang 1-7 10 mm. *: Compared with group A (TNF- α treated), the indicated protein expression has a statistically significant, $P < 0.05$.

Figure 1: Relative protein expression of MKK3, MKK4, MKK6 and PMKK4

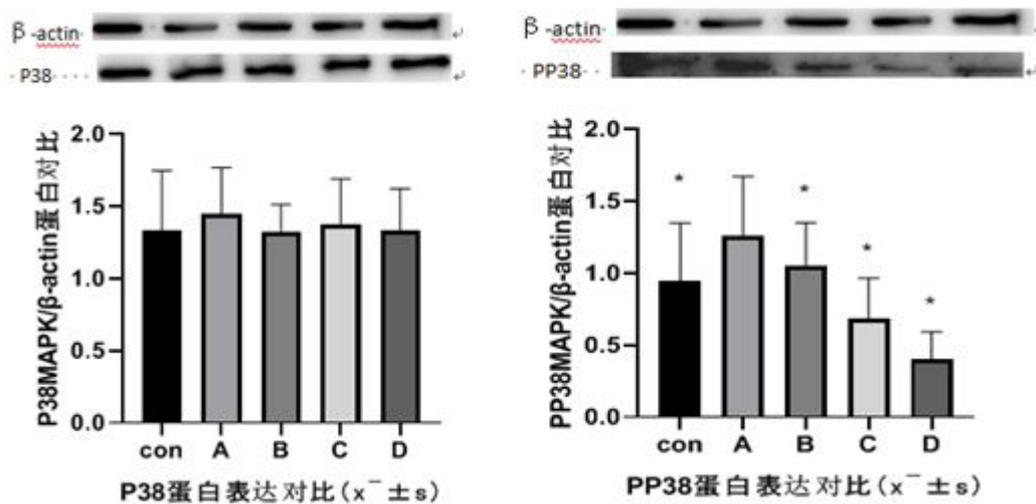
Relative protein expression of P38MAPK and PP38MAPK

Compared with A group, there was no significant difference in P38 protein expression after inflammatory factor (TNF- α) stimulation, but PP38 protein expression was significantly increased ($P < 0.05$). After the intervention of Ang 1-7, the protein expression of PP38 was significantly lower than that of A group ($P < 0.05$), but there was no significant difference in the reduction of the protein in group B. There was no significant difference in the protein expression of P38. The expression of PP38 protein in groups B, C, and D, E had a decreasing trend, but there was no significant difference ($P > 0.05$). There was significant differences of the protein expression of PP38 within multiple comparisons between group A and groups C and D ($P < 0.05$), and there was significant differences between group B and group D ($P < 0.05$). There was no significant difference of the protein expression of PP38 between group B and groups A and C (Table 2 and Figure 2).

Group	n	P38	PP38
A group (Control)	6	1.40±0.654	1.09±0.399*
B group (TNF-α)	6	1.53±0.704	1.53±0.525
C group (TNF-α + Ang 1-7 0.1mm)	6	1.35±0.509	1.13±0.254
D group (TNF-α + Ang 1-7 1 mm)	6	1.33±0.387	0.82±0.323*
E group (TNF-α + Ang 1-7 10 mm)	6	1.29±0.364	0.55±0.287*

*: Compared with TNF-α group, $P < 0.05$

Table 2: Comparison of P38 and PP38 protein expression in HL-I cells of each group ($x \pm s$)



Con: blank control group, group A: TNF-α treated, group B: TNF-α + Ang 1-7 0.1 mm, group C: TNF-α + Ang 1-7 1 mm, group D: TNF-α + Ang 1-7 10 mm. *: Compared with group A (TNF-α treated), the indicated protein expression has a statistically significant, $P < 0.05$

Figure 2: Relative protein expression of P38 and PP38

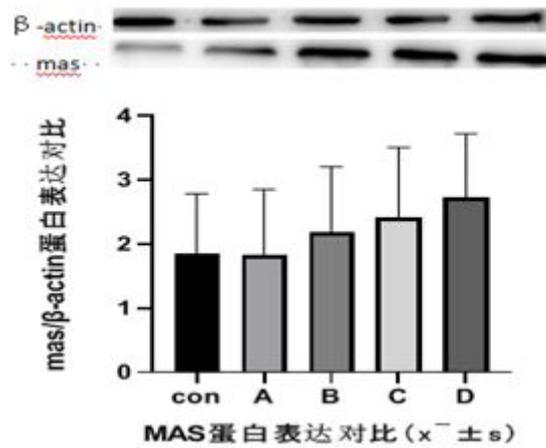
Expression of MAS receptor

Compared with A group, there was no significant difference in the protein expression of MAS after stimulation with inflammatory factor (TNF-α). After intervention with Ang 1-7, the protein expression of MAS was higher than that of B group and C group, but there was no significant difference ($P > 0.05$). The expression of MAS protein in groups C, D, and E showed an increasing trend, but there was no significant difference ($P > 0.05$). There was no significant difference of MAS protein expression in multiple comparisons within the groups ($P > 0.05$) (Table 3 and Figure 3).

Group	n	MAS
A group (Control)	6	1.72±0.985
B group (TNF-α)	6	1.78±0.977
C group (TNF-α + Ang 1-7 0.1mm)	6	2.10±0.928
D group (TNF-α + Ang 1-7 1 mm)	6	2.29±0.996
E group (TNF-α + Ang 1-7 10 mm)	6	2.54±1.007

*: Compared with TNF-α group, $P < 0.05$

Table 3: Comparison of MAS protein expression in HL-I cells of each group ($x \pm s$)



CON: blank control group, group A: TNF-α treated, group B: TNF- + Ang 1-7 0.1 mm, group C:TNF-α + Ang 1-7 1 mm, group D:TNF-α + Ang 1-7 10 mm. *: Compared with group A (TNF-α treated), the indicated protein expression has a statistically significant, $P < 0.05$

Figure 3: Relative protein expression of MAS

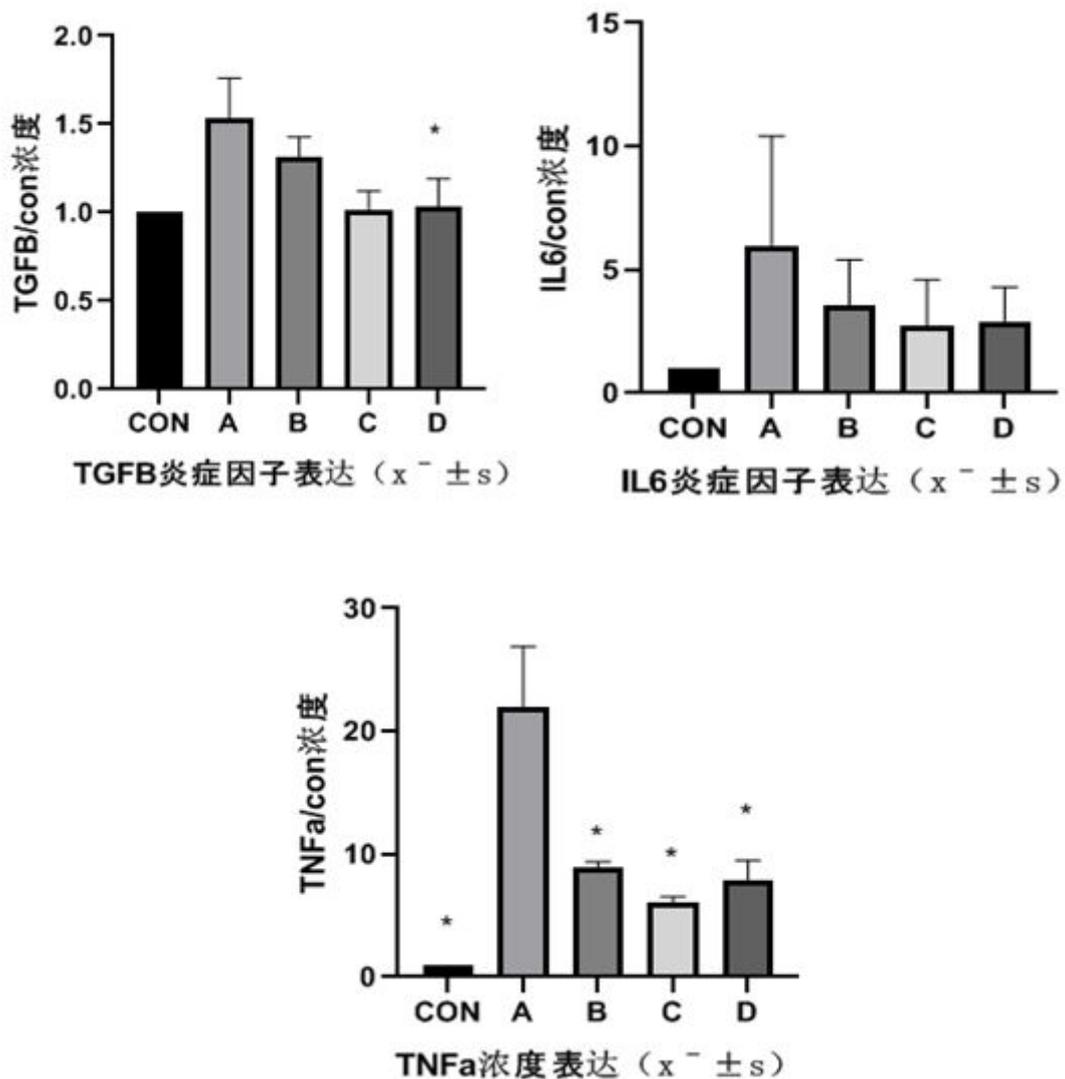
Flow multi-factor detection of the expression of inflammatory factors of TGF-β, IL-6, TNF-α

Compared with the A group, the TGF-β of group B was significantly increased after stimulating factor (TNF-α) was given, and it was statistically significant ($P < 0.05$). The expression of TGF-β was decreased after the intervention of Ang 1-7, and there was a significant difference in group E ($P < 0.05$). There is no significant difference between group C, D and group B ($P > 0.05$). There is no significant difference in multiple comparisons within the group between C,D and E($P > 0.05$) (Table 4 and Fig 4). Compared with A group, IL-6 in group B increased after stimulating factor (TNF-α) was given. The expression of TGF-β decreased after the intervention of Ang 1-7, ($P > 0.05$) (Table 4 and Fig 4). Compared with the A group, TNF-α in group B was significantly increased after stimulating factor (TNF-α) was given, and it was statistically significant ($P < 0.05$). The expression of TNF-α was significantly decreased after the intervention of Ang 1-7, and it was statistically significant ($P < 0.05$). However, there was no significant difference in multiple comparisons between groups C, D and E ($P > 0.05$) (Table 4 and Figure 4).

Group	n	TGF-β	IL-6	TNF-α
A group (Control)	6	1.00±0.00*	1.00±0.00	1.00±0.00*
B group (TNF-α)	6	1.47±0.204	5.96±3.961	21.12±4.907
C group (TNF-α + Ang 1-7 0.1mm)	6	1.30±0.092	3.65±1.633	8.64±0.747*
D group (TNF-α + Ang 1-7 1 mm)	6	1.09±0.156	2.76±1.656	6.07±0.404*
E group (TNF-α + Ang 1-7 10 mm)	6	1.19±0.216*	3.19±1.407	7.66±1.554*

*: Compared with TNF-α group, $P < 0.05$

Table 4: Comparison of TGF-β IL6 TNF-α protein expression in HL-I cells of each group(x±s)



Con: blank control group, group A: TNF- α treated, group B: TNF- α + Ang 1-7 0.1 mm, group C: TNF- α + Ang 1-7 1 mm, group D: TNF- α + Ang 1-7 10 mm. *: Compared with group A (TNF- α treated), the indicated protein expression has a statistically significant, $P < 0.05$

Figure 4: Relative protein expression of TGF- β IL-6 and TNF- α

Discussion

P38 kinase is a proline-mediated serine/threonine kinase of the mitogen-activated protein kinase (MAPK) family, which is activated by environmental stress and signal pathways. P38MAPKs (especially P38-a) are involved in cellular responses to stress, environmental and intracellular stresses at many levels, such as high osmotic pressure, oxidative stress, inflammation, DNA damage and other physiological conditions that involve cell changes [7]. P38 -a is involved in a variety of functions, and the disorder of this pathway is related to diseases such as inflammation, immune disorders or cancer [8]. The activation of P38 is mediated by the phosphorylation of specific regulatory tyrosine and threonine sites, and the three kinases MKK3, MKK4, and MKK6 are the upstream activators of P38 [9]. P38a regulates many functions of cardiomyocytes, including hypertrophy, contractility, fibrosis and apoptosis [10,11,12].

Atrial muscle fibrosis leads to local conduction slowdown and conduction disorders, leading to unidirectional conduction block [13]. Fibrosis increases the number of fibroblasts and change their characteristics, by changing the interaction between cardiomyocytes

and fibroblasts to couple the electrophysiological behavior of cardiomyocytes, thereby promoting atrial fibrillation [14]. The role of the RAAS system in the remodeling of atrial structure has been confirmed. Clinical studies have shown that RAAS inhibitors are benefit for patients with atrial fibrillation after pulmonary vein isolation [15]. RAAS inhibitors also inhibit atrial fibrosis and atrial remodeling, and delay atrial fibrillation [16,17]. Ang II is the central factor of RAAS, which causes vasoconstriction, increases myocardial afterload, promotes left ventricular hypertrophy, indirectly increases atrial pressure, and increases the ductility of myocardial cells [18]. In addition, Ang II increases oxidative stress, thereby inducing inflammatory collagen fiber deposition and causing atrial fibrosis. In animals with an overexpression of angiotensin converting enzyme, Ang II levels are significantly increased, and there was obvious atrial enlargement, atrial fibrosis and atrial fibrillation. Ang II promotes atrial remodeling through TGF- β /Smad2/3 signaling pathway [19]. MAPKs are a group of important downstream molecules of Ang II, which are involved in the increased expression of TGF- β 1 inducing by Ang II [20]. The increased expression of TGF- β 1 inducing by Ang II is the main mechanism of Ang II inducing atrial fibrosis. In a clinical study, 56 patients with rheumatic heart disease are divided into atrial fibrillation group and sinus rhythm group. Left atrial appendage tissue is collected during cardiac surgery to assess myocardial fibrosis. The study found that the atrial MAPKs activity in the atrial fibrillation group is significantly higher than sinus rhythm group, the atrial TGF- β 1 and CTGF mRNA and protein expression in patients with atrial fibrillation increases significantly, confirming that the MAPKs/TGF- β 1/TRAF6 signaling pathway is involved in the occurrence of atrial fibrosis in patients with atrial fibrillation [21,22]. In addition, the increased expression of TGF- β 1 induced by Ang II is the main mechanism of Ang II-induced myocardial fibrosis [23]. Ang II increased the activation of mouse fibroblast MAPKs, up-regulate the expression of TGF- β 1 and CTGF, and promote the proliferation of fibroblasts [24]. The increase in CTGF expression induced by Ang II or TGF- β 1 is an important factor involved in atrial fibrosis and atrial fibrillation. In vitro studies on mouse fibroblasts show that Ang II stimulates TGF- β 1 on atrial fibroblasts, which acts in a Smad-independent manner. In this study, it is also confirmed that the TGF- β 1/TRAF6 signaling pathway is involved in atrial fibrosis.

Transforming growth factor (TGF- β 1) is a key fibroblast growth factor. TGF- β 1 regulates cell proliferation, apoptosis and migration, and regulate the synthesis of extracellular matrix (such as up-regulating the expression of fibronectin and collagen fibers). Overexpression of TGF- β 1 may cause atrial fibrosis and atrial fibrillation. The increase in CTGF expression induced by Ang II or TGF- β is an important factor involved in atrial fibrosis and atrial fibrillation [25]. TGF- β may be the main switch that regulates the transition from inflammatory response to fibrosis [26]. Masaki Ikeuchi et al. show that early inhibition of TGF- β aggravates ventricular dysfunction and inflammatory response, while late destruction of TGF- β signaling protects interstitial fibrosis and hypertrophic remodeling [27]. Peter P R et al. show that although extensive inhibition of TGF- β after infarction lead to early death of heart rupture, the specific destruction of TGF- β receptors by cardiomyocytes has a protective effect and extensively stimulates anti-inflammatory and cytoprotective signals [28]. Therefore, the adverse effects of early TGF- β inhibition on infarcted myocardium may not be due to the direct effect of cardiomyocyte survival, but reflect the loss of anti-inflammatory effects of inflammatory cells, endothelial cells or fibroblasts [29].

This study confirmed that inflammatory stress triggers the MKK-P38MAPKs signaling pathway, which increased the expression of MKK3, MKK4, MKK6, and PMKK4 proteins, and the expression of phosphorylated PP38 protein was also significantly increased, and the concentration of inflammatory factors TGF β also increased. Thus, these changes may cause myocardial fibrosis leading to the occurrence and maintenance of atrial fibrillation.

When a large number of myocardial cells are suddenly damaged after myocardial infarction, resulting in the formation of collagen scars [30]. Necrotic cells released dangerous signals, which activate innate immune pathways, and trigger a strong inflammatory response. Downstream signals focus on the activation of mitogen-activated protease (MAPK) and NF- κ B. These pathways drive the expression of pro-inflammatory genes including inflammatory factors (such as TNF- α , IL-1 β , IL-6 and IL-18) [31-33]. Inflammation signals promote the adhesion between leukocytes and endothelial cells, leading to extravasation of neutrophils and monocytes. When the infiltrating white blood cells clear the necrotic cells, mediators that inhibit inflammation are released [34,35]. The inhibition of inflammatory response is related to the activation of repair cells, leading to the proliferation of fibroblasts to maintain the integrity of the infarcted ventricle [36-38]. TNF- α is an important factor in vascular inflammation, and its level is elevated in vascular diseases. Many effects of TNF- α are similar to Ang II. Arenas et al. report that Ang II regulates endothelial cells secreting

inflammatory cytokines TNF- α and matrix metalloproteinase-2 (MMP) [39]. Ang II stimulates the production of TNF- α through a PKC-dependent pathway in macrophages [40]. In monocytes, macrophages, vascular smooth muscle cells and endothelial cells, TNF- α activates NF- κ B, thereby inducing the production of adhesion molecules and chemokines, such as IL-6 and IL-8 [41]. Cytokines also play an important role in the occurrence and development of atherosclerotic lesions [42]. The level of IL-18 expressed in atherosclerotic lesions is elevated. Sahar et al. prove that IL-18 activates Src, PKC, and MAPK. In Ang II stimulated smooth muscle cells, IL-18 is enhanced by activating NF- κ B, and Ang II also induces IL-18 receptor mRNA expression through STAT3 [43]. Nami K et al. use Ang II to stimulate the HL-1 cell line. Ang II induces reactive oxygen species (ROS) production and activates MAPK, TGF- β 1, IL-6, IL-1 β , NF- κ B, and TNF- α . Ang II regulates atrial fibrillation through inflammatory mechanisms and MAPK signaling pathways produced ROS [44,45]. In addition, TNF- α , IL-6, and IL-1 β are also the prototype stress activators of P38-a. Cytokines bind to different types of surface receptors to determine the p38-a phosphorylation pathway, usually with TRAF ubiquitin ligase and TAK1 and others with MAPK3. The P38-a-MK2 pathway regulates the expression of TNF- α and mediated the production of TNF- α induced pro-inflammatory factors, while limits TNF- α induced apoptosis.

This study confirmed that the administration of inflammatory stimuli (TNF- α) has an effect through the MKK-P38-MAPKs signaling pathway. The expression of inflammatory factors was as follows: TGF- β and TNF- α were significantly increased and statistically significant, and IL-6 level was also increased, but there was no significant difference.

The formation and degradation mechanism of angiotensin II (Ang II) is an important factor that determines its ultimate physiological effect. Ang II is an octapeptide. Angiotensin is cut into angiotensin I by aspartase renin, and angiotensin I is converted into Ang II by angiotensin-converting enzyme (ACE) [46]. A recently study discovers carboxypeptidase ACE2 cleaves an amino acid from Ang I or Ang II, reduces the level of Ang II and increases the vasodilatory metabolite Ang 1-7 [47]. ACE2/Ang1-7/MAS axis regulation regulates fiber generation and remodeling. In another study, male rats are divided into sham operation group, Ang II group, Ang II + Ang 1-7 group, Ang II + Ang 1-7 + A77 group, stimulate for 4 weeks, and finally tissues are collected. Results indicate that chronic Ang 1-7 prevents cardiomyocyte hypertrophy and interstitial fibrosis induced by hypertension. Ang 1-7 acts directly on the heart tissue. It is also confirmed that the anti-fibrosis and anti-hypertrophy effects of Ang 1-7 are not mediated by changes in the number of AT1 or AT2 cardiac receptors [48]. In a mouse model of asthma, it is also confirmed that Ang 1-7 inhibits ovalbumin-induced airway leukocyte influx, perivascular and peribronchial inflammation, fibrosis, and goblet cell hyperplasia or metaplasia [49]. The ACE2/Ang1-7/MAS axis regulates the recruitment and activation of leukocytes. In the model of pulmonary hypertension, the activation of the ACE2/Ang1-7/MAS axis also regulates the expression of pro-inflammatory factors, reducing the expression of TNF- α , TGF- β , IL-6, IL-1 β and increases the expression of anti-inflammatory factor IL-10 [50,51]. Jun Mori implants micro-osmotic pumps in male db/db mice (diabetic cardiomyopathy mice) at the age of 5 months, and gives them Ang 1-7 for 28 days. It is found that Ang 1-7 inhibits the increase of myocardial protein kinase C level and the loss of extracellular signal-regulated kinase 1/2 phosphorylation, and reduces the levels of triglyceride and ceramide in the heart of db/db mice, and increases the expression of triglyceride lipase in myocardial fat [52].

In this study, the protein expressions of MKK3, MKK4, MKK6, PMMK4, and PP38 were significantly increased after inflammatory factor (TNF- α) stimulation, and after intervention with Ang₁₋₇, the protein expression of MKK3, MKK4, MKK6, PMMK4, and PP38 was significantly lower than the stimulation group ($P < 0.05$). In terms of inflammatory factors, TGF- β and TNF- α were significantly increased after TNF- α stimulation, and the expression of TGF- β and TNF- α was significantly reduced after Ang₁₋₇ intervention ($P < 0.05$). IL-6 also had the same effects as TGF- β and TNF- α trend, but there was no statistically significant.

Conclusion

Ang1-7 through MKK/P38MAPKs inflammatory signal pathway protected on TNF- α stimulated mouse HL-1 cells

Conflicting of Interest

All authors declare that have no financial or non-financial competing interests in relation to our work. Such as 1 .Receiving reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of the manuscript, either now or in the future.2 .Holding stocks or shares in an organization that may in any way gain or lose financially from the publication of the manuscript, either now or in the future.3 .Holding, or currently applying for, patents relating to the content of the manuscript.4 .Receiving reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript.

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All authors declare that we have no Authors from pharmaceutical companies, or other commercial organizations

All authors declare that we have no Editorial Board Members and Editors in relation to our work.

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