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Telmisartan inhibits Ang II-induced MMP-9 expression in macrophages in stabilizing atheromatous plaque

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Abstract. – **OBJECTIVE:** **To investigate the effects of telmisartan on matrix metalloproteinase-9 (MMP-9) expression in macrophages induced by angiotensin II (Ang II) and its mechanism.**

MATERIALS AND METHODS: **THP-1 cells were adopted for research, and phorbol-12-myristate-13-acetate (PMA) was utilized to induce THP-1 cells to be transformed into macrophages, with Ang II as a stimulating factor and telmisartan as a therapeutic drug. Cell counting kit-8 (CCK8) and lactate dehydrogenase (LDH) were applied to detect cell viability and toxicity. Enzyme-linked immunosorbent assay (ELISA) was performed to measure the MMP-9 release level. Polymerase Chain Reaction (PCR) and Western blotting were conducted to detect the expressions of MMP-9 messenger ribonucleic acid (mRNA) and protein, respectively. The mechanism of action was further studied, and the activity of cyclooxygenase-2 (COX2)/macrophage-expressed gene 1 (mPEG1) pathway was determined via PCR and Western blotting.**

RESULTS: **The 1 mM Ang II could remarkably activate the synthesis and release of MMP-9 as well as the COX2/mPEG1 pathway in macrophages. However, telmisartan could effectively repress the Ang II-induced MMP-9 synthesis and release in the macrophages, and suppress the COX2/mPEG1 pathway in the macrophages activated by Ang II.**

CONCLUSIONS: **Telmisartan can inhibit the activation of MMP-9 in the macrophages by suppressing the COX2/mPEG1 pathway.**

Key Words:
Angiotensin II, MMP-9, Macrophages, Telmisartan, COX2/mPEG1.

Introduction

Angiotensin II (Ang II), as a major component of renin-angiotensin system (RAS), plays its role by virtue of specific seven transmembrane Ang II receptors which are divided into two subtypes, namely angiotensin II type 1 (AT1) and AT2, and Ang II mainly perform its functions via the AT1 receptor in the cardiovascular system. Studies have proven that Ang II plays an important role in atherosclerosis (AS)[1,2], and it is not only a vasoactive hormone that regulates blood pressure, aldosterone release, and sodium and water reabsorption, but also a powerful pro-inflammatory cytokine[3]. Ang II regulates the expressions of many substances, including growth factors, cytokines, chemical factors, and adhesion molecules, which are related to cell growth/apoptosis, fibrosis, and inflammation[4,5]. The role of Ang II in inducing inflammations may be its primary mechanism of participating in AS and acute coronary syndrome (ACS). Ang II can damage the anti-fibrinolytic function of endothelial cells and increase the adhesion molecules expressed in the endothelial cells. It can also induce inflammatory responses in the vessel wall, promote circulating monocytes to enter the tunica intima and accelerate the macrophages to transform into foam cells[6]. In addition, in the early stage of AS, Ang II can stimulate smooth muscle cells to produce monocyte chemotactic protein-1 (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1), which are helpful for mononuclear macrophages to adhere to the vessel wall, phagocytize oxidized low-density lipoprotein (oxLDL) to form foam cells and accelerate the occurrence and development of AS lesions[7]. Several studies[8] have demonstrated that macrophages, as major inflammatory cells that are involved in AS plaque, play a vital role in the process of the AS plaque rupture. The activated macrophages can secrete cytokines, promote the degradation and apoptosis of smooth

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muscle cell and decline the ability to synthesize and repair extracellular matrix. They can also secrete matrix metalloproteinases (MMPs), degrade extracellular matrix and decrease the intensity of fibrous caps. MMPs are a category of proteolytic enzymes closely correlated with plaque instability and rupture, which are mainly synthesized in the macrophages. MMPs synthesized by the macrophages in local plaque are the most crucial enzymes for plaque instability[9]. Studies have illustrated that the macrophages in unstable plaque can induce the generation of MMPs through cyclooxygenase-2 (COX2)/prostaglandin E2 (PGE2) pathway[10]. It is manifested through research that MMP-9 is a primary matrix proteinase causing plaque instability as well as one of the major factors reflecting plaque stability[11]. Some clinical studies[12] have revealed that angiotensin receptor blocker (ARB) agents cannot only lower the blood pressure, but also decrease the occurrence rate of cardiovascular events in the patients. It suggests that ARB agents may exert effects in the process of stabilizing plaques. In this experiment, human monocytic cell line THP-1 cells were adopted to observe the impacts of telmisartan, a type of ARB agent, on the AT1 and AT2 receptors as well as MMP-9 expression in mononuclear macrophages at the cellular level.

Materials and Methods

Cells and Materials

The monocytic cell line THP-1 cells were purchased from the Cell Center of the Institute of Basic Medical Sciences of the Chinese Academy of Medical Sciences (Beijing, China). Dulbecco's Modified Eagle Medium (DMEM) was bought from Gibco (Rockville, MD, USA), AT1 and MMP-9 antibodies were purchased from CST (Danvers, MA, USA), enzyme-linked immunosorbent assay (ELISA) kit was provided by R&D Systems (Minneapolis, MN, USA), Polymerase Chain Reaction (PCR) kit was purchased from Beyotime, and fluorescent antibody was bought from Abcam (Cambridge, MA, USA).

Cell Culture

The cell suspension was transferred into a 10 mL centrifuge tube and added with 5 mL DMEM containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA). After centrifugation at 1,000 rpm for 10 min, the supernatant was discarded, and an appropriate amount of DMEM containing 10% FBS was added, pipetted and mixed evenly, so as to adjust the cell density to 1×10^5/mL. Then, the cells were seeded into a 25 mL culture flask for culture in an incubator with 5% CO_2 at 37°C, and the medium was replaced after 24 h. 100 ng/mL phorbol-12-myristate-13-acetate (PMA) was applied to induce differentiation for 48 h, and the differentiation level was determined under a light microscope.

Polymerase Chain Reaction (PCR)

The treated cells in each group were collected to extract the total ribonucleic acid (RNA) using TRIzol (Invitrogen, Carlsbad, CA, USA). After the concentration of the samples was measured, the reverse transcription system was added according to the concentration to perform the reverse transcription. The former 40 cycles were utilized to synthesize the complementary deoxyribonucleic acid (cDNA), and the reverse transcription reaction conditions were set for PCR amplification. Real-time fluorescence signal was collected after each cycle, and the amplification and melting curves were recorded.

ELISA

The cell suspension was aspirated into an Eppendorf (EP) tube, followed by centrifugation at 12,000 rpm for 5 min, collection of supernatant and preservation in a refrigerator at -20°C for detection. ELISA was performed to measure the MMP-9 concentration in the supernatant. The operations were conducted in strict accordance with the steps in the instructions of the kits and microplate reader. All the reagents, working standard solution and samples were prepared. The bands with excessive plate frames were removed, which were placed in a foil pouch with desiccant, and then the pouch was sealed. 100 μL diluent RD1-34 was added into each well. Then, 100 μL of standard substance or sample was added into each well, which was covered by adhesive tapes. After that, the plate was placed on a horizontal shaker at (500±50) rpm for incubation at room temperature for 2 h. The substances in each well were aspirated and washed for 4 times. 200 μL affinity MMP-9 was added into all wells to detect the antibodies. Next, the plate was sealed with a new piece of sealing paper, which was washed again after incubation on the shaker at room temperature for 1 h. Then, 200 μL substrate solution was added into each well, followed by incubation away from the light on the horizontal shaker at 500±50 rpm for 30 min. After that, 50 μL stop

buffer was added into each well. The optical density at 450 nm was read, and that at 570 nm was calibrated for every well using the microplate reader within 30 min. The MMP-9 concentration was calculated on the basis of the MMP-9 standard curve.

Western Blotting

Cells in each group were fetched and washed twice with D-Hank's solution, which was then absorbed clearly using absorbent paper. Next, 150 μL ice-cold lysis buffer was added into each group and then placed on the ice for lysis for 30 min. The proteins in each group were collected into an EP tube using a cell scraper, followed by centrifugation at 12,000 rpm and 4°C. After that, the supernatant was sucked and transferred into a new EP tube, and 5× loading buffer was added and mixed after the protein concentration was determined via bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA), followed by heating at 100°C for 6 min. Then, 30 μL proteins were added into prepared separation gel and spacer gel loading wells, which were subjected to electrophoresis in the electrophoresis buffer under a proper voltage. After that, the gel was stuck closely to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA), followed by transfer in transmembrane solution at 0°C under a constant voltage of 100 V for 60 min. After the PVDF membrane was blocked in 5% skim milk powder at room temperature for 1 h, it was clipped according to the molecular weight and then blocked in primary antibodies in the refrigerator at 4°C overnight. The PVDF membrane was taken out the next day and rinsed with Tris-Buffered Saline-Tween 20 (TBST), followed by addition of secondary antibody immunoglobulin G (IgG) (1:5000) for incubation at room temperature for 1 h. After that, the membrane was rinsed with TBST again, and Tanon 5200 immunofluorescence development system was applied for development as well as measurement and calculation of grayscale.

Statistical Analysis

The results were presented as mean ± variance, and Statistical Product and Service Solutions (SPSS) 16.0 software (SPSS Inc., Chicago, IL, USA) was utilized for statistical processing and

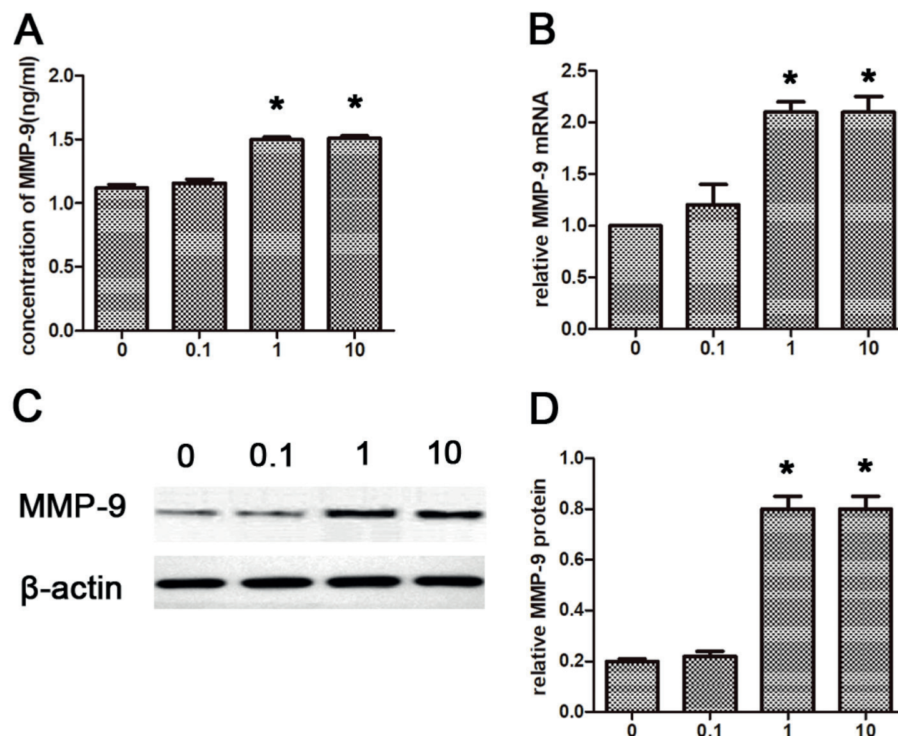

Figure 1. Activation of MMP-9 in macrophages *via* Ang II. ***A,*** MMP-9 release levels induced by different concentrations of Ang II (0.1 μM, 1 μM and 10 μM) in the macrophages detected by ELISA. ***B,*** MMP-9 messenger RNA (mRNA) synthesis induced by different concentrations of Ang II (0.1 μM, 1 μM and 10 μM) in the macrophages detected by PCR. ***C,*** MMP-9 protein synthesis induced by different concentrations of Ang II (0.1 μM, 1 μM and 10 μM) in the macrophages detected by Western blotting. ***D,*** Analysis of grayscale value of Western blotting for MMP-9 protein in each group. *$p<0.05$ *vs.* blank group

analysis of the data. Homogeneity of variance test was performed for all the data measured, and one-way analysis of variance was adopted for comparison among groups, followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ suggested that the difference was statistically significant.

Results

Ang II Induced MMP-9 Expression and Release in the Macrophages

The Ang II-induced (0.1 μM, 1 μM and 10 μM) MMP-9 expressions in the macrophages were detected via ELISA, PCR and Western blotting. According to ELISA, compared with that in control group, MMP-9 release in the macrophages was not significantly increased by 0.1 μM Ang II, but remarkably increased by 1 μM and 10 μM Ang II (Figure 1A, $p<0.05$). However, there were no differences among the groups. The PCR results indicated that the MMP-9 RNA level in macrophages could be elevated through the action of Ang II. In comparison with control group, 1 μM and 10 μM Ang II had statistically significant differences in the MMP-9 RNA level (Figure 1B, $p<0.05$), while the difference between the two groups was not significant. The MMP-9 protein expressed in the macrophages was detected, and it was revealed that the changes were consistent with those of MMP-9 RNA. After the action of 1 μM and 10 μM Ang II, the MMP-9 protein expressions in the macrophages were increased, with significant differences compared with those in control group (Figure 1C and 1D, $p<0.05$), but there was no difference between 1 μM and 10 μM Ang II. Ang II could not only induce the MMP-9 RNA and protein expressions in the macrophages, but also increase the MMP-9 release.

Ang II Induced COX2/Macrophage-Expressed Gene 1 (mPEG1) Activation in Macrophages

The Ang II-induced (0.1 μM, 1 μM and 10 μM) COX2/mPEG1 expressions in the macrophages were detected by means of PCR and Western blotting. The results manifested that the RNA levels of COX2 and mPEG1 in the macrophages could be enhanced after the action of Ang II. Statistically significant differences were observed between 1 μM and 10 μM Ang II and control group (Figure 2A and 2B, $p<0.05$), while the difference between 1 μM and 10 μM Ang II was not significant. Similarly, it was discovered through protein level examination that the expressions of COX2 and mPEG1 proteins were improved after Ang II treatment, of which more prominent elevation was detected after the action of 1 μM and 10 μM Ang II, displaying differences compared with those in control group (Figure 2C, 2D and 2E, $p<0.05$). These results suggest that Ang II is capable of inducing the activation of the COX2/mPEG1 pathway, activating the RNA expressions as well as protein synthesis of COX2 and mPEG1.

Telmisartan Exerted a Toxic Effect on Macrophages

The toxic effects of different concentrations of telmisartan on the macrophages were detected via cell counting kit-8 (CCK8) and lactate dehydrogenase (LDH). CCK8 results showed that compared with control group, 0.1 μM and 1 μM telmisartan could not remarkably inhibit the cell proliferation, but 10 μM telmisartan had prominent inhibitory effects on the cell proliferation (Figure 3A, $p<0.05$). LDH release test indicated that 10 μM telmisartan produced more notable cytotoxicity than control group, and the LDH released by cells were increased evidently (Figure 3B, $p<0.05$). However, no significant cytotoxicity was produced by 0.1 μM and 1 μM telmisartan. Therefore, the experiments were performed using telmisartan at experimental doses of 0.1 μM and 1 μM.

Telmisartan Inhibited the Increase in MMP-9 Release and Expression in the Macrophages Induced by Ang II

The levels of MMP-9 release and expression in the macrophages in blank control group, Ang II group and telmisartan (0.1 μM and 1 μM) + Ang II group were determined by virtue of ELISA, PCR and Western blotting. ELISA indicated that MMP-9 release in the macrophages was suppressed significantly by 0.1 μM and 1 μM telmisartan compared with those in Ang II group (Figure 4A, $p<0.05$). There were no significant differences between groups with different concentrations of telmisartan. The results of PCR and Western blotting revealed that telmisartan could repress the MMP-9 RNA level and protein synthesis in the macrophages, showing significant differences compared with those in Ang II group (Figure 4B, 4C and 4D, $p<0.05$). The differences between groups with different concentrations of telmisartan were not significant. Those results imply that telmisartan can inhibit Ang II in inducing MMP-9 release and expression in the macrophages and decrease the expression and release of MMP-9 in the macrophages.

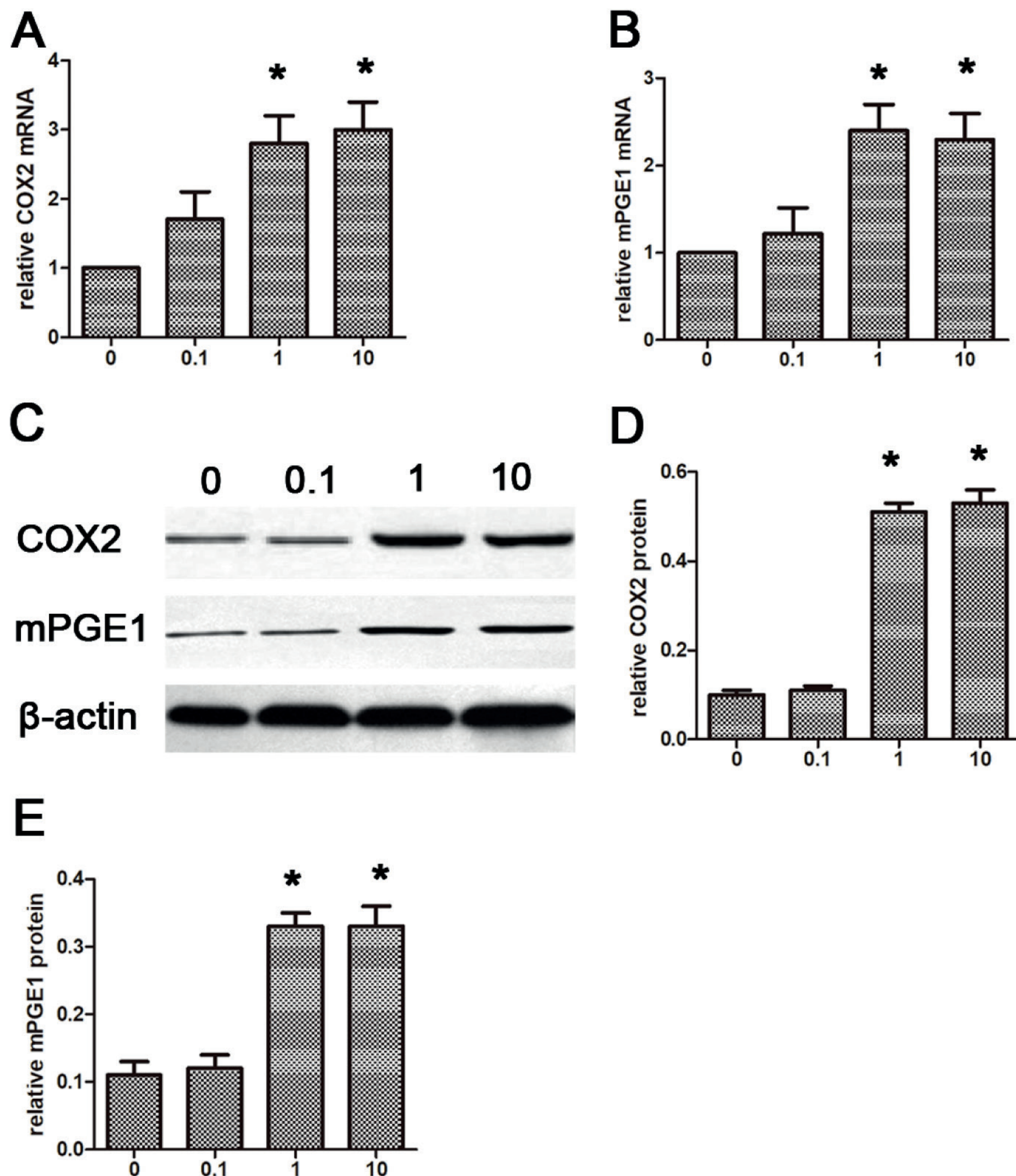

Figure 2. Activation of COX2/mPEG1 pathway in the macrophages *via* Ang II. ***A,*** COX2 mRNA levels induced by different concentrations of Ang II (0.1 μM, 1 μM and 10 μM) in the macrophages detected *via* PCR. ***B,*** MPEG1 mRNA levels induced by different concentrations of Ang II (0.1 μM, 1 μM and 10 μM) in the macrophages detected *via* PCR. ***C,*** COX2 and mPEG1 protein expressions induced by different concentrations of Ang II (0.1 μM, 1 μM and 10 μM) in the macrophages detected *via* Western blotting. ***D,*** Analysis of grayscale value of Western blotting for COX2 protein in each group. ***E,*** Analysis of grayscale value of Western blotting for mPEG1 protein in each group. *$p<0.05$ *vs.* blank group

Telmisartan Inhibited COX2/mPEG1 Expression in the Macrophages Induced by Ang II

The COX2/mPEG1 expression in the macrophages in blank control group, Ang II group and telmisartan (0.1 μM and 1 μM) + Ang II group were measured through PCR and Western blotting. It was shown that in comparisons with those in Ang II group, the RNA levels of COX2 and mPEG1 in the macrophages were inhibited markedly by 0.1 μM and 1 μM telmisartan, with statistically significant differences. However, there were no differences between groups with different concentrations of telmisartan (Figure 5A, 5B). Similarly, it was discovered through protein level examination that the expressions of COX2 and mPEG1 proteins were suppressed after treatment with telmisartan, displaying differences

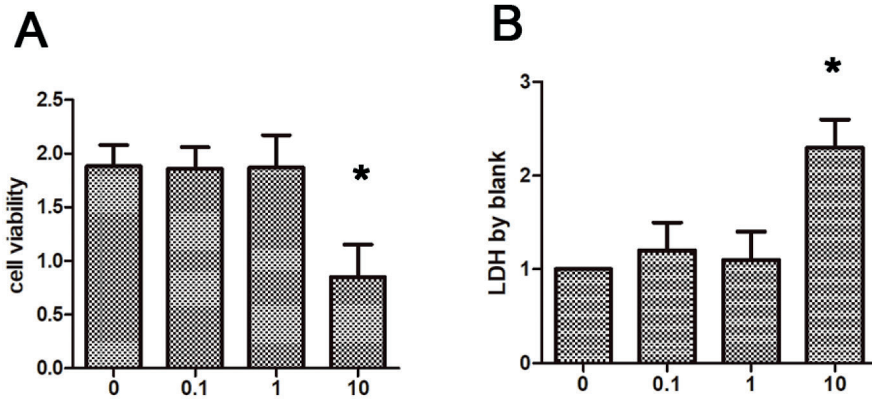

Figure 3. Effects of telmisartan on the macrophage viability. ***A,*** Changes in the macrophage viability after treatment with different concentrations of telmisartan (0.1 μM, 1 μM and 10 μM) detected *via* CCK8. ***B,*** Cytotoxicity in the macrophages after treatment with different concentrations of telmisartan (0.1 μM, 1 μM and 10 μM) detected *via* LDH. *p<0.05 *vs.* blank group

compared with those in Ang II group (Figure 5C, 5D and 5E, p<0.05). All these suggest that telmisartan can inhibit the activation of COX2/mPEG1 pathway induced by Ang II and suppress the synthesis of COX2 and mPEG1 proteins.

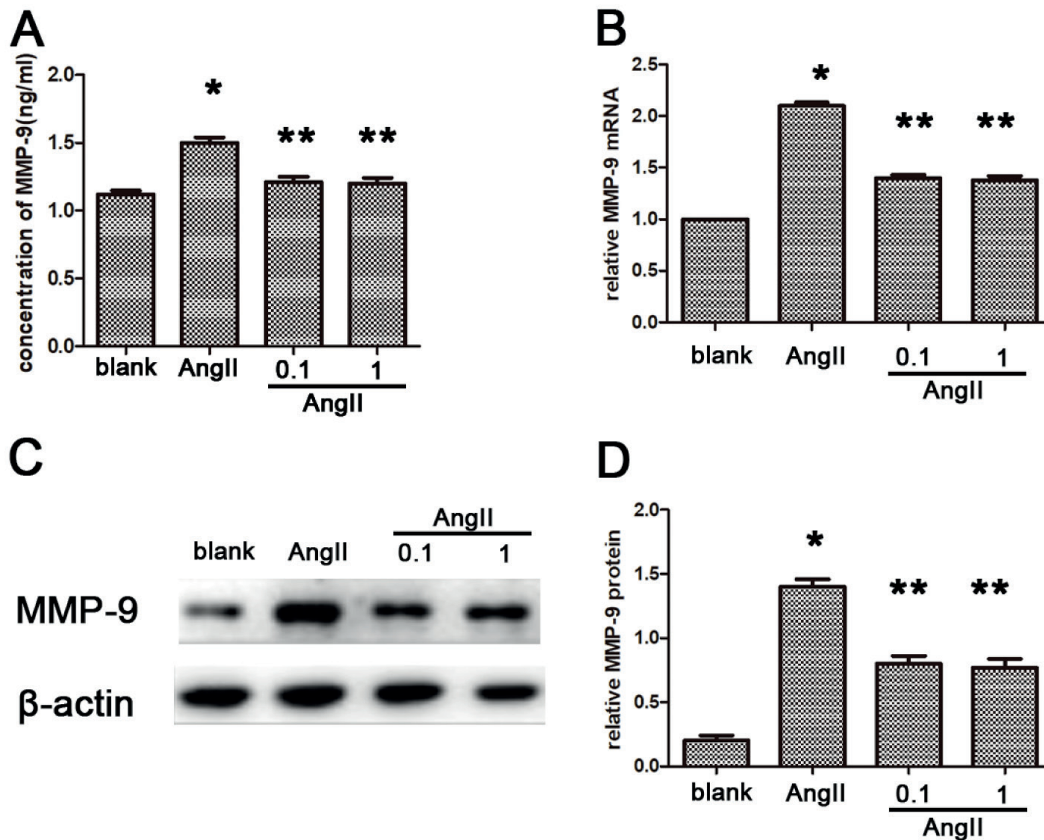

Figure 4. Telmisartan inhibits MMP-9 activity in the macrophages. ***A,*** Levels of MMP-9 release in the macrophages in blank control group, Ang II group and telmisartan (0.1 μM and 1 μM) + Ang II group detected *via* ELISA. ***B,*** MMP-9 mRNA synthesis in the macrophages in blank control group, Ang II group and telmisartan (0.1 μM and 1 μM) + Ang II group deteced *via* PCR. ***C,*** MMP-9 protein synthesis in the macrophages in blank control group, Ang II group and telmisartan (0.1 μM and 1 μM) + Ang II group detected *via* Western blotting. ***D,*** Analysis of grayscale value of Western blotting for MMP-9 protein in each group. *p<0.05 *vs.* blank group, **p<0.05 *vs.* Ang II group

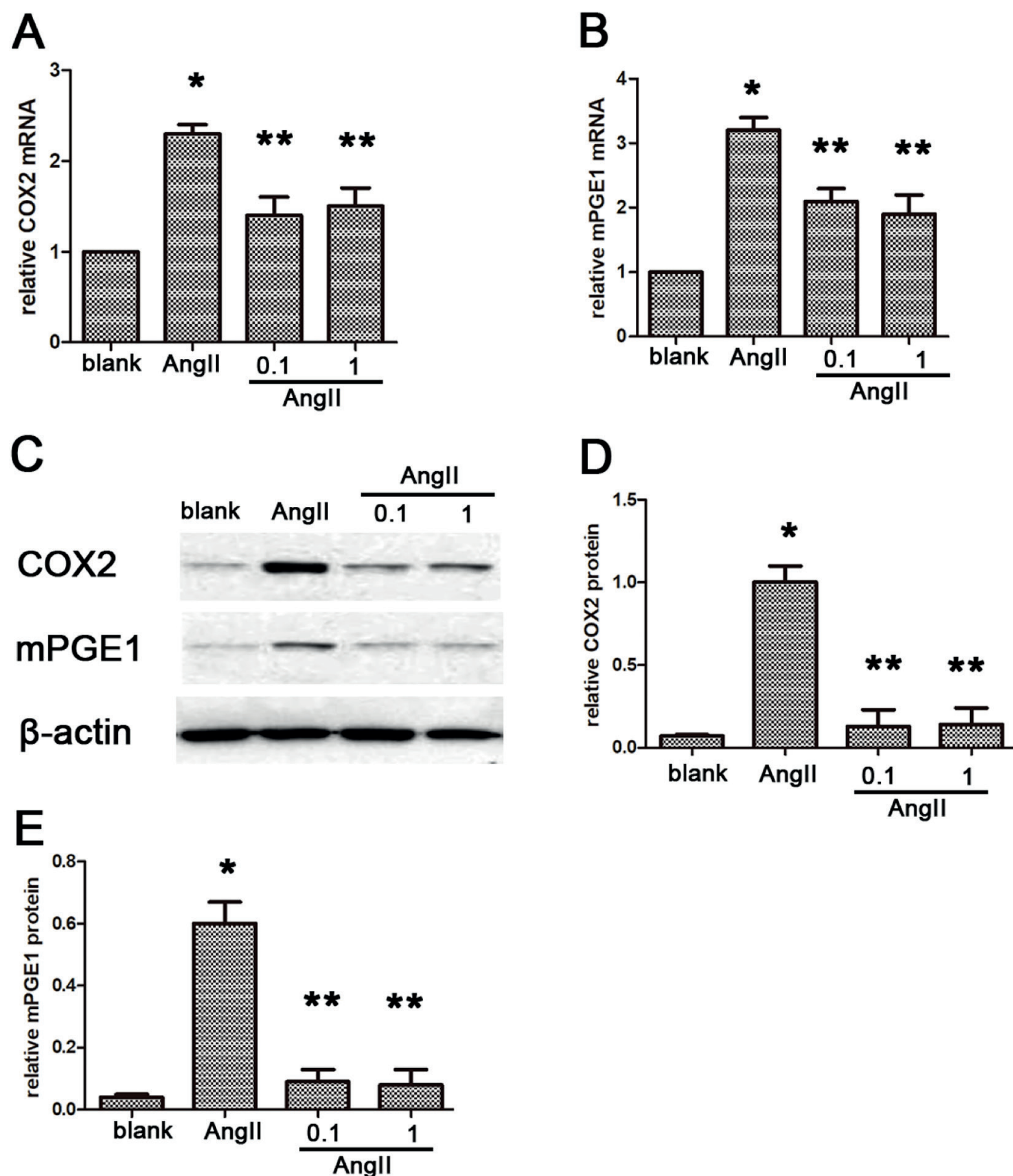

Figure 5. Inhibitory effect of telmisartan on COX2/mPEG1 pathway in the macrophages. ***A,*** COX2 mRNA levels in the macrophages in blank control group, Ang II group and telmisartan (0.1 μM and 1 μM) + Ang II group detected *via* PCR. ***B,*** MPEG1 mRNA levels in the macrophages in blank control group, Ang II group and telmisartan (0.1 μM and 1 μM) + Ang II group detected *via* PCR. ***C,*** COX2 and mPEG1 protein expressions in the macrophages in blank control group, Ang II group and telmisartan (0.1 μM and 1 μM) + Ang II group detected via Western blotting. ***D,*** Analysis of grayscale value of Western blotting for COX2 protein in each group. ***E,*** Analysis of grayscale value of Western blotting for mPEG1 protein in each group. *$p<0.05$ *vs.* blank group, **$p<0.05$ *vs.* Ang II group.

Discussion

THP-1 cells were adopted in this research, and PMA was utilized to induce THP-1 cells to be transformed into the macrophages. According to the results of this experiment, the MMP-9 and COX2/mPEG1 expressions in the macrophages cultured *in vitro* were increased under the induction of Ang II, while the Ang II-induced MMP-9 synthesis and release in the macrophages could be efficiently inhibited by telmisartan. Such an inhibitory effect may be generated by the

activation of the COX2/mPEG1 pathway in the macrophages.

Atherosclerosis is a common pathological basis of cardiovascular and cerebrovascular diseases, which is one of the most important reasons threatening human health at present. ACS is a major manifestation of cardiac events in patients with coronary heart disease, of which the common pathophysiologic basis is that the coronary AS plaques become unstable and rupture due to multiple factors, thus leading to thrombosis. Epidemiological studies[13,14] have manifested that the RAS is an independent risk factor for AS plaque rupture and ACS. Further analyses have revealed that Ang II in the RAS can increase inflammatory factors, such as interleukin-6 (IL-6), tumor necrosis factor (TNF), and IL-1, in the vascular tissues through several pathways[15]. These inflammatory factors can aggravate platelet aggregation, stimulate MMPs production and promote proliferation of smooth muscle cells, ultimately increasing the plaque instability and inducing plaque rupture[16].

The proinflammatory effect of Ang II is achieved mainly by stimulating the macrophages. In turn, the activated macrophages can secrete large quantities of inflammatory factors, block the apoptosis and proliferation of smooth muscle cells and damage the synthesis and repair ability, thereby injuring the integrity of vessel wall[17]. Also, the macrophages can secrete the hydrolase MMPs. According to the research of Moreau et al[18], the expressions of MMP-1, MMP-3, and MMP-9 were elevated markedly in the cultured human macrophages after induction with oxLDL. Among them, the MMP-9 is a crucial predictor of plaque stability. In clinical observation of the MMP-9 expression during coronary atherectomy, the positive expression of MMP-9 exists in 83% coronary atherosclerotic plaques of patients with unstable angina and in 25% plaques of patients with stable angina. However, the positive expression is not detected in the normal internal mammary artery without any lesion[19]. The MMP-9 in those unstable plaques are generated by the macrophages through the COX2/PGE2 pathway[10]. It was discovered in this study that telmisartan could effectively suppress the Ang II-induced MMP-9 synthesis and release in the macrophages and inhibit the COX2/mPEG1 expression at the same time. The results prove that telmisartan can protect the plaque stability and reduce the plaque rupture, which may serve as an important target for the treatment of coronary heart disease.

Conclusions

We showed that Telmisartan can suppress the activation of MMP-9 in the macrophages induced by Ang II, which is realized possibly by repressing the COX2/PGE2 pathway in the cells.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Durante A, Peretto G, Laricchia A, Ancona F, Spartera M, Mangieri A, Cianflone D. Role of the renin-angiotensin-aldosterone system in the pathogenesis of atherosclerosis. Curr Pharm Des 2012; 18: 981-1004.
2) Ruiz-Ortega M, Lorenzo O, Suzuki Y, Ruperez M, Egido J. Proinflammatory actions of angiotensins. Curr Opin Nephrol Hypertens 2001; 10: 321-329.
3) Phillips MI, Kagiyama S. Angiotensin II as a pro-inflammatory mediator. Curr Opin Investig Drugs 2002; 3: 569-577.
4) Sadoshima J. Cytokine actions of angiotensin II. Circ Res 2000; 86: 1187-1189.
5) Li AL, Lv JB, Gao L. MiR-181a mediates Ang II-induced myocardial hypertrophy by mediating autophagy. Eur Rev Med Pharmacol Sci 2017; 21: 5462-5470.
6) Kalupahana NS, Moustaid-Moussa N. The renin-angiotensin system: a link between obesity, inflammation and insulin resistance. Obes Rev 2012; 13: 136-149.
7) Usui M, Egashira K, Tomita H, Koyanagi M, Katoh M, Shimokawa H, Takeya M, Yoshimura T, Matsushima K, Takeshita A. Important role of local angiotensin II activity mediated via type 1 receptor in the pathogenesis of cardiovascular inflammatory changes induced by chronic blockade of nitric oxide synthesis in rats. Circulation 2000; 101: 305-310.
8) Galis ZS, Sukhova GK, Kranzhofer R, Clark S, Libby P. Macrophage foam cells from experimental atheroma constitutively produce matrix-degrading proteinases. Proc Natl Acad Sci U S A 1995; 92: 402-406.
9) Welgus HG, Campbell EJ, Cury JD, Eisen AZ, Senior RM, Wilhelm SM, Goldberg GI. Neutral metalloproteinases produced by human mononuclear phagocytes. Enzyme profile, regulation, and expression during cellular development. J Clin Invest 1990; 86: 1496-1502.
10) Cipollone F, Prontera C, Pini B, Marini M, Fazia M, De Cesare D, Iezzi A, Ucchino S, Boccoli G, Saba V, Chiarelli F, Cuccurullo F, Mezzetti A. Overexpression of functionally coupled cyclooxygenase-2 and prostaglandin E synthase in symptomatic atherosclerotic plaques as a basis of prostaglan-

din E(2)-dependent plaque instability. Circulation 2001; 104: 921-927.

11) Crisby M, Nordin-Fredriksson G, Shah PK, Yano J, Zhu J, Nilsson J. Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: implications for plaque stabilization. Circulation 2001; 103: 926-933.

12) Lindholm LH, Ibsen H, Dahlof B, Devereux RB, Beevers G, de Faire U, Fyhrquist F, Julius S, Kjeldsen SE, Kristiansson K, Lederballe-Pedersen O, Nieminen MS, Omvik P, Oparil S, Wedel H, Aurup P, Edelman J, Snapinn S. Cardiovascular morbidity and mortality in patients with diabetes in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet 2002; 359: 1004-1010.

13) Alderman MH, Madhavan S, Ooi WL, Cohen H, Sealey JE, Laragh JH. Association of the renin-sodium profile with the risk of myocardial infarction in patients with hypertension. N Engl J Med 1991; 324: 1098-1104.

14) Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, Et A. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 1992; 359: 641-644.

15) Schieffer B, Schieffer E, Hilfiker-Kleiner D, Hilfiker A, Kovanen PT, Kaartinen M, Nussberger J, Harringer W, Drexler H. Expression of angiotensin II and interleukin 6 in human coronary atherosclerotic plaques: potential implications for inflammation and plaque instability. Circulation 2000; 101: 1372-1378.

16) Biasucci LM, Vitelli A, Liuzzo G, Altamura S, Caligiuri G, Monaco C, Rebuzzi AG, Ciliberto G, Maseri A. Elevated levels of interleukin-6 in unstable angina. Circulation 1996; 94: 874-877.

17) Shah PK, Falk E, Badimon JJ, Fernandez-Ortiz A, Mailhac A, Villareal-Levy G, Fallon JT, Regnstrom J, Fuster V. Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. Circulation 1995; 92: 1565-1569.

18) Moreau M, Brocheriou I, Petit L, Ninio E, Chapman MJ, Rouis M. Interleukin-8 mediates downregulation of tissue inhibitor of metalloproteinase-1 expression in cholesterol-loaded human macrophages: relevance to stability of atherosclerotic plaque. Circulation 1999; 99: 420-426.

19) Brown DL, Hibbs MS, Kearney M, Loushin C, Isner JM. Identification of 92-kD gelatinase in human coronary atherosclerotic lesions. Association of active enzyme synthesis with unstable angina. Circulation 1995; 91: 2125-2131.