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## Chronic angiotensin-(1–7) administration improves vascular remodeling after angioplasty through the regulation of the TGF- $\beta$ /Smad signaling pathway in rabbits <sup>☆</sup>

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## ABSTRACT

**Objective:** Angiotensin-(1–7) [ANG-(1–7)] has been reported to attenuate neointimal formation after vascular injury and stent implantation in rats, but the mechanism remains mostly unresolved. Interestingly, the levels of circulating transforming growth factor-beta1 (TGF- $\beta$ 1) after myocardial infarction were suppressed by ANG-(1–7), which suggests a possible downstream target for the anti-remodeling action of ANG-(1–7). Our study focused on the effects of ANG-(1–7) on vascular remodeling, including neointimal formation and collagen synthesis, and determining whether or not these effects were dependent upon the TGF- $\beta$  signaling pathway.

**Methods:** Thirty-two New Zealand white rabbits underwent sham surgery or angioplasty in abdominal aorta. The animals were divided into four groups, which were sham, control, ANG-(1–7), and ANG-(1–7) + A-779. Subsequently, an osmotic minipump was implanted to deliver saline, ANG-(1–7) (576  $\mu\text{g kg}^{-1} \text{d}^{-1}$ ) or ANG-(1–7) + A-779 (576  $\mu\text{g kg}^{-1} \text{d}^{-1}$ ) for 4 weeks.

**Results:** The ANG-(1–7) group displayed a significant reduction in neointimal thickness (207.51  $\pm$  16.70  $\mu\text{m}$  vs. 448.08  $\pm$  15.30  $\mu\text{m}$ ,  $P < 0.001$ ), neointimal area (0.266  $\pm$  0.009  $\text{mm}^2$  vs. 0.408  $\pm$  0.002  $\text{mm}^2$ ,  $P < 0.001$ ), and restenosis rate (28.13  $\pm$  2.74% vs. 40.13  $\pm$  2.74%,  $P < 0.001$ ) when compared to the control group. ANG-(1–7) also inhibited collagen synthesis by significantly decreasing the mRNA expression of *Collagen I* and *Collagen III* (vs. Control group: 0.2190  $\pm$  0.0036 vs. 0.3852  $\pm$  0.0212,  $P < 0.001$  and 1.1328  $\pm$  0.0554 vs. 1.7378  $\pm$  0.1164,  $P < 0.001$ , respectively). Furthermore, the expression of TGF- $\beta$ 1 and phosphor-Smad2 (p-Smad2) were significantly suppressed by ANG-(1–7) (vs. Control group: 1.21  $\pm$  0.07 vs. 1.54  $\pm$  0.08,  $P < 0.001$  and 0.31  $\pm$  0.01 vs. 0.43  $\pm$  0.02,  $P < 0.001$ , respectively), but no effect on p38 phosphorylation was observed. [D-Ala<sup>7</sup>]-ANG-(1–7) (A-779), showed a tendency to attenuate the anti-remodeling effects of ANG-(1–7).

**Conclusion:** ANG-(1–7) decreases the amount of vascular remodeling, including a reduction in neointimal formation and collagen synthesis, after angioplasty in rabbits. The responsible mechanism may function through the possible down-regulation of TGF- $\beta$ 1 levels and inhibition of the Smad2 pathway.

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## Introduction

Coronary reperfusion treatment is widely used to restore blood flow to the ischemic myocardium. Vascular remodeling has been

**Abbreviations:** ANG, angiotensin; TGF- $\beta$ 1, transforming growth factor-beta1; A-779, [D-Ala<sup>7</sup>]-Angiotensin-(1–7); p-Smad2, phosphor-Smad2; VSMCs, vascular smooth muscle cells; RAS, renin-angiotensin system; ECM, extracellular matrix; ACE-I, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor antagonist; ISR, in-stent restenosis; ARCF, adult rat cardiac fibroblasts; HE, hematoxylin and eosin; IEL, interal elastic lamina; EEL, external elastic lamina.

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identified as the major cause for the delayed failure of angioplasty procedures [1]. Vascular remodeling may result from abnormal wound healing, including initial constriction, neointimal formation, and extracellular matrix (ECM) accumulation [2]. Experiments in humans and animal models have indicated that the renin-angiotensin system (RAS) plays a very important role in vascular remodeling [3]. RAS intervention, through Angiotensin converting enzyme inhibitors (ACE-I) and angiotensin receptor antagonist (ARB), has been proven to be a rather safe and effective method for the treatment of cardiovascular diseases [4,5]. Therefore, targeting the RAS-axis may provide more potent therapies for the inhibition of vascular remodeling after angioplasty.

Angiotensin-(1–7) [ANG-(1–7)] is an endogenous bioactive peptide in the RAS. It is considered to have a counteracting effect to

angiotensin II (ANG II) on the cardiovascular system by its inhibition of cell migration and proliferation, ECM deposition, inflammation, thrombosis [6]. Most effects are beneficial to the prevention of vascular remodeling. Furthermore, Langeveld et al. [7] and Strawn et al. [8] demonstrated that continuous ANG-(1–7) infusion attenuates neointimal formation after vascular injury and reduced in-stent restenosis (ISR) in a rat model, respectively. No further investigation has been undertaken to observe effects of ANG-(1–7) on collagen synthesis, which is another important aspect that affects vascular remodeling after angioplasty. In addition, the mechanism by which ANG-(1–7) prevents remodeling after vascular injury has not yet been studied in detail. Over the past few years, there has been an increased focus on the mechanism for the anti-remodeling action of ANG-(1–7) in heart. Iwata et al. [9] demonstrated that ANG-(1–7) could decrease transforming growth factor-beta 1 (TGF- $\beta$ 1) mRNA levels in cultured adult rat cardiac fibroblasts (ARCF). Grobe et al. [10] recently demonstrated that the plasma levels of TGF- $\beta$ 1 in myocardial infarction rat models were 40% lower in the group with ANGII co-infused with ANG-(1–7) than in the group with ANGII alone. Taken together, these observations reveal a trend towards a suppression in the expression levels of TGF- $\beta$ 1 by ANG-(1–7) during the remodeling phase in heart, which suggests a possible mechanism for the anti-remodeling activity of ANG-(1–7). The vascular response to angioplasty and the response of the myocardium to infarction are very similar, with an early accumulation of myofibroblasts and a subsequent loss of cells that results in an acellular matrix-rich structure. Given this similarity, we hypothesized that the anti-remodeling action of ANG-(1–7) during vascular remodeling after angioplasty may be associated with the TGF- $\beta$ 1 signaling pathway.

TGF- $\beta$  belongs to a superfamily of proteins that serve critical roles in ECM production and the regulation of cell growth, differentiation, migration, and apoptosis in different organ systems. TGF- $\beta$ 1, which is the most important isoform for the cardiovascular system has been reported to be a most potent profibrotic cytokine [11]. Moreover, many studies [12] found that TGF- $\beta$  expression is increased in human restenotic lesions as well as in neointimal hyperplasia after balloon injury in animals. In experimental models [13–15], targeting TGF- $\beta$  by antibody, soluble receptor or ribozyme oligonucleotides effectively reduced neointimal formation and the constrictive remodeling that are associated with angioplasty. The mechanisms involved in TGF- $\beta$ -mediated vascular remodeling are complex, including the activation of Smad protein, protein kinases-production of mediators [16].

In the present study, ANG-(1–7) was given for 4 weeks to a rabbit abdominal aorta injury model to explore the effects of chronic ANG-(1–7) administration on vascular remodeling after angioplasty. Furthermore, the anti-remodeling response to ANG-(1–7) was characterized by examining collagen synthesis and the expression levels of the protein components of the TGF- $\beta$  signaling pathway, including those in the Smad-dependent (Smad2) and Smad-independent (p38) pathway. The resulting data allowed us to determine that the mechanism of the anti-remodeling activity of ANG-(1–7) after angioplasty may depend on the inhibition of the TGF- $\beta$ /Smad signaling pathway and ECM deposition.

## Materials and methods

**Animals.** The experimental population consists 32 healthy, 12-week-old New Zealand white rabbits weighing 1.7–2.2 kg, which were derived from the Animal Experiment Centre of Guangdong Province, China and were raised in the Department of Animal Experiment Center of Sun Yat-sen University. All animals were provided with normal rabbit chow and tap water and housed in individual cages with a 12-dark/light cycle. All animal experimental

procedures had ethical approval and followed the Guidelines for Animal Care Issued by the First Affiliated Hospital of Sun Yat-sen University.

**Experimental protocol.** All rabbits were randomly [17] divided into four groups with eight rabbits each: sham group, control group, ANG-(1–7) group, and ANG-(1–7) + A-779 group (Table 1).

The day before surgery, each rabbit received 50 mg aspirin. Each rabbit was then anesthetized with 3% pentobarbital sodium (1 ml/kg). The right femoral was exposed and punctured with needle to obtain a pulsatile blood flow. Then a wire was cannulated into the artery. A 4F sheath was introduced into the right femoral artery and passed to the abdominal artery via the wire. A 200 IU heparin-sodium solution was administered through the sheath. A 3.5 × 23-mm angioplasty balloon catheter was advanced over a standard 0.014-in flexible guide-wire in the abdominal aorta. Artery wall injury was achieved by withdrawing the catheter through the abdominal aorta 3 times while the catheter balloon was inflated to 15 atmospheric pressures. A 1-min reperfusion followed each inflation. After the surgery, 5000 IU heparin-sodium was administered by intravenous injection. Each rabbit in the sham group was similarly exposed as a sham-operated control.

An osmotic minipump was implanted subcutaneously on the back between the shoulder blades and the hips. The pumps were used to deliver ANG-(1–7) (576  $\mu$ g kg<sup>-1</sup> d<sup>-1</sup>, in saline) to the ANG-(1–7) and ANG-(1–7) + A-779 groups, A-779 (576  $\mu$ g kg<sup>-1</sup> d<sup>-1</sup>, in saline) to the ANG-(1–7) + A-779 group or saline alone (5  $\mu$ l h<sup>-1</sup>) to the sham and control groups into the jugular vein [7,8].

Blood pressure (BP) and heart rate (HR) were measured on a multi-channel electrophysiological recorder before and after the surgery.

Aspirin was administered orally at a dose of 50 mg/d for 4 weeks, and gentamicin was administered by intramuscular injection at a dose of 80,000 IU/d for 3 days.

**Angiography.** Following 4 weeks of treatment, animals were anesthetized by intravenous injection of 3% pentobarbital sodium (1 ml/kg). Abdominal angiography was done through the left femoral.

**Histological analysis.** After angiography, rabbits were killed by an overdose of pentobarbital. Abdominal aortas were then partially perfusion-fixed, impregnated in 4% formalin, harvested, fixed, embedded in paraffin, sectioned at 5  $\mu$ m and stained with hematoxylin and eosin (HE) or Weigert for histological analysis. Single sections from each animal were viewed and photographed with an Olympus digital camera. Cross-sectional areas and diameters were determined at 4× magnification, using 3–4 sections per vessel, with the Image-Pro Plus Program 6.0. Neointima was defined as the area delimited by the internal elastic lamina (IEL) and the lumen artery wall interface. Media was defined as the area circumscribed by the IEL and external elastic lamina (EEL). The following equations were used: (1) neointimal thickness = (IEL circumference – lumen circumference)/2 $\pi$ ; (2) media thickness = (EEL circumference – IEL circumference)/2 $\pi$ ; (3) neointimal area = IEL area – lumen area; and (4) stenosis rate = neointimal area/(lumen area + neointimal area) × 100%.

**Gene expression by RT-PCR.** Total RNA was extracted using a commercially available kit (Takara, Japan). cDNA was obtained by

**Table 1**

Body weight (kg) of rabbit before and 4 weeks after surgery in each group.

Group	Number	Before surgery	Four weeks after surgery
Sham group	8	2.02 ± 0.21	2.88 ± 0.25
Control group	8	1.98 ± 0.19	2.85 ± 0.27
ANG-(1–7) group	8	2.00 ± 0.23	2.92 ± 0.30
ANG-(1–7) + A-779 group	8	2.08 ± 0.19	2.93 ± 0.24

reverse transcription at 42 °C for 60 min and 70 °C for 15 min. The *Collagen I* and *Collagen III* genes were amplified using specific conditions (the reaction included the oligonucleotides listed in Supplement 1).

The thermal profile used on a Bio-Rad thermal cycle consisted of 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C (*Collagen I*) or 60 °C (*Collagen III*, *GAPDH*), for 45 s and extension at 72 °C for 1 min.

RT-PCR products were resolved on 2.5% agarose gels in 1× Tris–borate-EDTA (TBE) buffer, visualized by ethidium bromide, photographed using a gel 1000 UV documentation system (Bio-Rad, Hercules, CA) and analyzed by densitometry using ImageJ software. The relative amount of each mRNA was normalized to the amount of the mRNA for house-keeping gene, *GAPDH*. Each sample was run and analyzed at least three times.

**Western blot analysis of protein.** Rabbits were perfused with saline, and the abdominal aorta was harvested, homogenized using a commercially available kit (Kangchen, China). After normalizing for the protein concentration of the lysate supernatants, equal amounts of protein were separated using 10% SDS–polyacrylamide gel electrophoresis (PAGE) and transferred onto poly-vinylidene difluoride (PVDF) membranes (Millipore, U.S.) and blocked for 1 h at room temperature in blocking solution (5% skim milk in TBST). The immunoblots were incubated with agitation at 4 °C overnight in the presence of specific antibodies directed against TGF-β1, Smad2, p-Smad2, p38, and phospho-p38 (Santa Cruz, U.S.) at dilutions of 1:200, 1:1500, 1:1000, 1:800, 1:400, respectively, in 5% BSA. After washing in TBST solution, the blots were further incubated for 1 h at room temperature with horseradish peroxidase-conjugated anti-mouse secondary antibody diluted 1:5000 (Boster, China) in the blocking solution. The blots were then washed three times in TBST, and antibody-bound protein was visualized with the Enhanced Chemiluminescence (ECL) kit (Beyotime, China). *GAPDH* was used as a house-keeping protein for the purposes of normalization and was analyzed following the same procedure as above, using a specific anti-*GAPDH* rabbit monoclonal antibody (KangCheng, China) at 1:5000 in 5% BSA. Signals were quantified by densitometry using the ImageJ Software.

**TGF-β1 in plasma assays.** Before, 1 week and 4 weeks after the surgery, blood (2 ml) was collected from the ear marginal vein and placed into an EP tube containing 40 μl aprotinin and 30 μl heparin-sodium. Blood samples were centrifuged at 4 °C at 3000 r/min for 15 min. The plasma was stored at –20 °C. The plasma levels of TGF-β1 were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (USCN life corporation, U.S.), following the manufacturer's instructions. For each experiment, a TGF-β1 standard curve was constructed using varying concentrations of recombinant human TGF-β1 protein, and a curve-fitting software program was used to quantify the concentration of TGF-β1 protein.

**Statistical analysis.** The SPSS 11.0 statistical package was used for all calculations. All data are presented as means ± standard deviation ( $\bar{x} \pm s$ ). All quantitative data were tested for homogeneity of variance and normality. Statistical differences were evaluated by one-way ANOVA, the ANOVA for repeated measures and LSD-t test, with the criterion for statistical significance set at  $P < 0.05$ .

## Experimental results

### Effects of chronic treatment with ANG-(1–7) on blood pressure and heart rate

Chronic ANG-(1–7) injection had no effect on BP or HR in rabbits after balloon injury. The systolic BP (SBP), diastolic BP (DBP) and HR ( $96 \pm 9$  mm Hg,  $63 \pm 7$  mm Hg, and  $343 \pm 35$  bpm, respectively,  $n = 8$ ) in the ANG-(1–7) group rabbits were not different

from those of the control group ( $93 \pm 13$  mm Hg,  $62 \pm 8$  mm Hg, and  $370 \pm 29$  bpm, respectively,  $n = 8$ ) (Supplement 2).

### Angiography result and effects of ANG-(1–7) on neointimal formation

After the intravenous infusion of ANG-(1–7) for four weeks, the vascular wall in the ANG-(1–7) group was smoother than that of the control group, as seen by abdominal angiography (Supplement 3).

Vascular injury caused notable formation of neointima in both the control group and ANG-(1–7) group. Chronic intravenous infusion with ANG-(1–7) significantly reduced the neointimal formation. When compared to the control group, the neointima in the ANG-(1–7) group had significantly decreased thicknesses, areas and restenosis rates ( $207.51 \pm 16.70$  μm vs.  $448.08 \pm 15.30$  μm,  $P < 0.001$ ;  $0.266 \pm 0.009$  mm<sup>2</sup> vs.  $0.408 \pm 0.002$  mm<sup>2</sup>,  $P < 0.001$ ;  $28.13 \pm 2.74\%$  vs.  $40.13 \pm 2.74\%$ ,  $P < 0.001$ , respectively). In contrast, there was no difference in the media thickness between the control and ANG-(1–7) groups. Neointimal thickness, area and restenosis rate in the ANG-(1–7) + A-779 group were not significantly different from those of the control group (Fig. 1).

Based upon the combination of the angiography images and the HE-stained sections, the neointima appeared to cause the formation of an irregular lumen. The cellular density of neointima in the control and ANG-(1–7) group was equally reduced (Supplement 4).

### Effects of chronic treatment with ANG-(1–7) on collagen synthesis

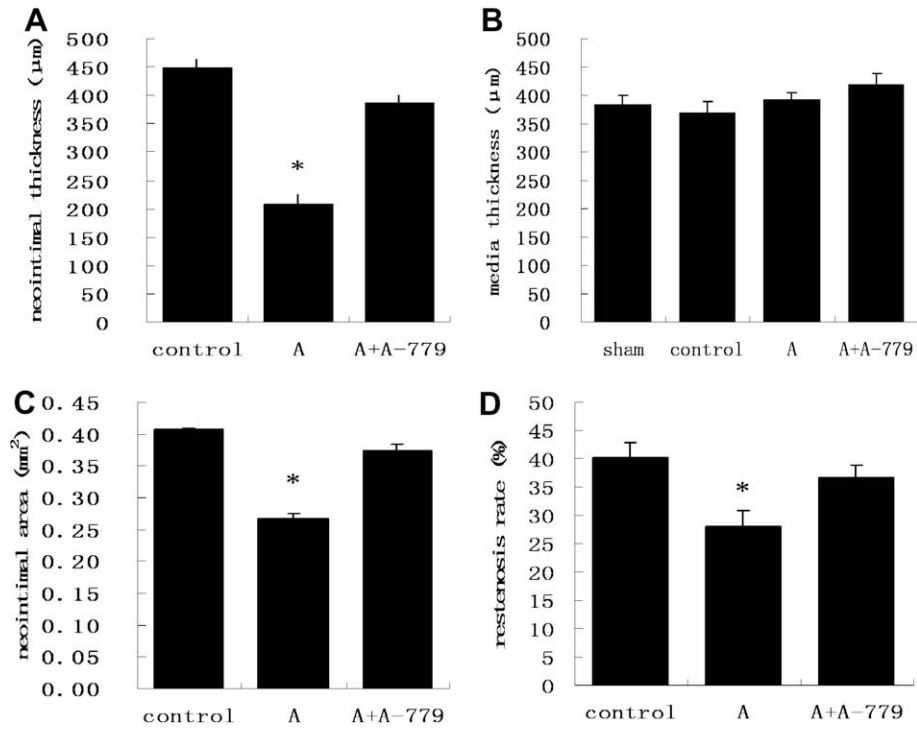
The expression levels of *Collagen I* and *Collagen III* were both significantly elevated in the control and ANG-(1–7) groups compared to the sham group (*Collagen I*:  $1.7378 \pm 0.0554$  and  $1.1328 \pm 0.1164$ , respectively, vs.  $0.4425 \pm 0.0459$ ,  $P < 0.001$ ; *Collagen III*:  $0.3852 \pm 0.0212$  and  $0.2190 \pm 0.0036$ , respectively, vs.  $0.1632 \pm 0.0057$ ,  $P < 0.001$ ). Despite this observation, ANG-(1–7) still has strong effects on reducing collagen synthesis. When compared to the control group, ANG-(1–7) significantly decreased the expression of *Collagen I* ( $P < 0.001$ ) and *Collagen III* ( $P < 0.001$ ) mRNA. However, A-779 diminished the effects of ANG-(1–7) on anti-collagen synthesis (Fig. 2).

### Effects of chronic treatment with ANG-(1–7) on circulating TGF-β1

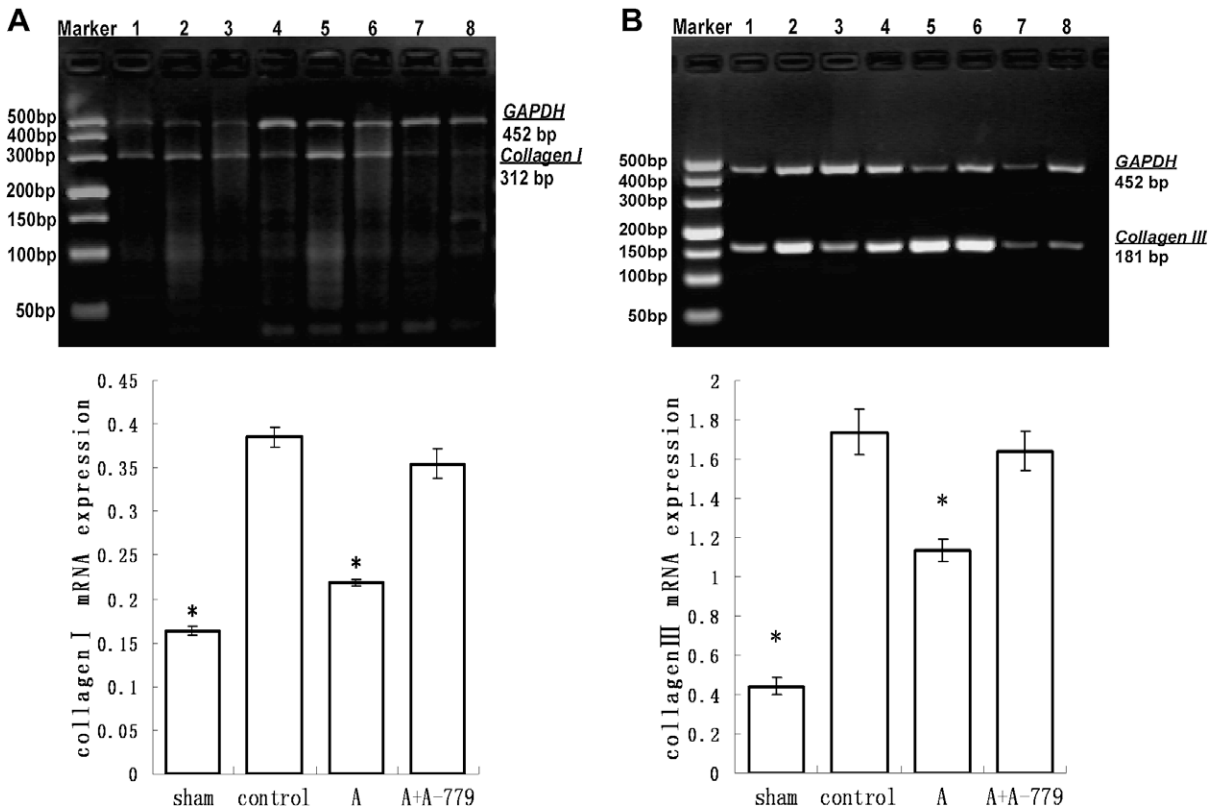
Compared to the sham group, the circulating levels of TGF-β1 were significantly elevated in the control and ANG-(1–7) group one week after the surgery (vs. sham group:  $2.53 \pm 0.35$  ng/ml and  $2.41 \pm 0.32$  ng/ml, respectively, vs.  $1.85 \pm 0.29$  ng/ml,  $P < 0.001$ , and  $P < 0.005$ , respectively). There was no significant difference between the control and ANG-(1–7) groups ( $P > 0.40$ ).

### Effects of chronic treatment with ANG-(1–7) on TGF-β signaling pathway

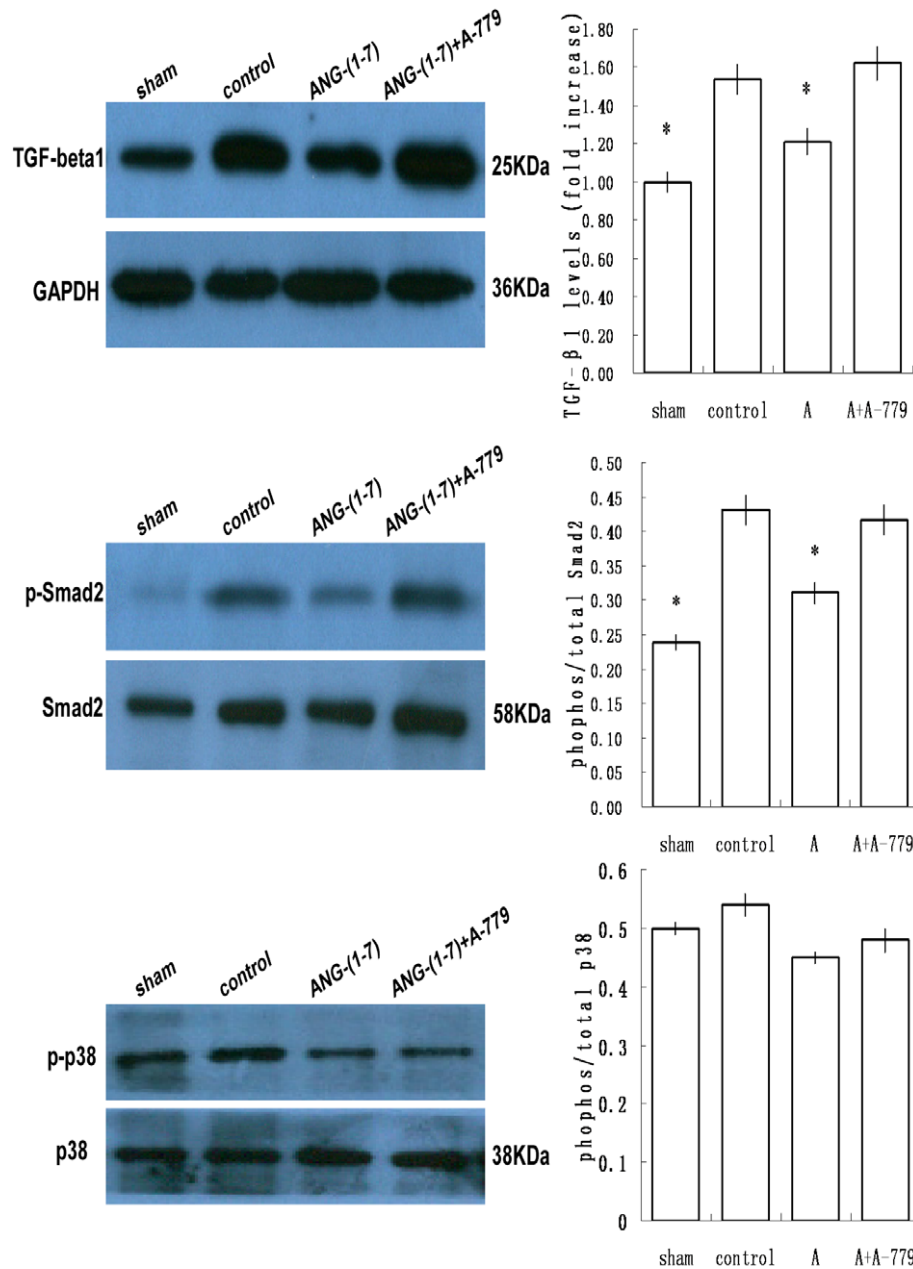
The Western blot analyses suggested that the expression of TGF-β1 protein in the injured vascular wall was still high four weeks after balloon injury (control group:  $1.54 \pm 0.08$ ; ANG-(1–7) group:  $1.21 \pm 0.07$ ; ANG-(1–7) + A-779 group:  $1.62 \pm 0.09$ ). Chronic ANG-(1–7) treatment resulted in an approximate 25% decrease in TGF-β1 protein levels when compared with the control group ( $P < 0.001$ ). Protein levels of p-Smad2 was also 1.80-, 1.30- and 1.75-fold increased in the control, ANG-(1–7) and ANG-(1–7) + A-779 groups, respectively, compared to sham group after 4 weeks of treatment ( $0.43 \pm 0.02$ ,  $0.31 \pm 0.01$ , and  $0.42 \pm 0.02$  vs.  $0.24 \pm 0.01$ , respectively;  $P < 0.01$ ). No effect was observed on the levels of p-p38. (Fig. 3).



**Fig. 1.** Sham, sham group; control, control group; A, ANG-(1-7) group; A + A-779, ANG-(1-7) + A-779 group. Data were expressed as means ± SD. One-way ANOVA and LSD-t were used to tested statistical differences. *P* < 0.001, vs. control group.



**Fig. 2.** Expression of *Collagen I* and *Collagen III* in rabbit abdominal aorta. Marker: DL500 DNA marker; lanes 1 and 2: cDNA from ANG-(1-7) + A-779 group rabbits; lanes 3 and 4: cDNA from ANG-(1-7) group rabbits; lanes 5 and 6: cDNA from control group rabbits; lanes 7 and 8: cDNA from sham group rabbits. The relative amount of each mRNA was normalized to the house-keeping gene, *GAPDH* mRNA. *P* < 0.001, vs. control group.



**Fig. 3.** The effect of chronic treatment with ANG-(1-7) on the expression TGF- $\beta$ 1, p-Smad2, and p-p38, as depicted by representative Western blots. The abdominal aorta was harvested following a four-week treatment with ANG-(1-7). TGF- $\beta$ 1 and p-Smad2 were significantly decreased by ANG-(1-7) compared with the control group ( $P < 0.05$ ). No effect was observed on the levels of p-p38.  $P < 0.001$ , vs. control group.

## Discussion

Following a four-week intravenous infusion ANG-(1-7) effectively reduced neointimal growth after angioplasty in a rabbit model. The abdominal angiography showed that the residual lumen was better preserved in the ANG-(1-7) group than in the control group. Furthermore, the HE and Weigert stains showed that ANG-(1-7) significantly reduced the neointimal thickness, neointimal area and restenosis rates when compared with those of the control group. The specific antagonist for ANG-(1-7), A-779, nearly diminished the anti-remodeling effects of ANG-(1-7). Taken together, these data demonstrated that ANG-(1-7) could alleviate neointimal formation after vascular injury and the anti-remodeling effects of ANG-(1-7) are thought to be mediated by its specific receptor Mas.

The effect of ANG-(1-7) on the TGF- $\beta$  system was also explored. Multiple lines of evidence [18] indicate that TGF- $\beta$  plays a critical role in vascular remodeling by inducing increases in the production and secretion of ECM and by regulating cell growth, differentiation, and migration. TGF- $\beta$  has been noted to exert biological effects through a Smad-dependent or a Smad-independent signaling pathway. Smad signaling plays a central role in nearly all transcriptional responses [16]. A relationship between the Smads and ECM synthesis has been demonstrated in vascular models. In addition, increased Smad staining in human fibrous atherosclerotic plaques has been found [19].

The use of balloon catheter denudation in rabbits up-regulated the expressions of TGF- $\beta$ 1 and p-Smad2, but not of p-p38. Furthermore, chronic ANG-(1-7) administration appears to have the potential to down-regulate components of an activated TGF- $\beta$



system after angioplasty in the rabbit abdominal aorta. One effect of this chronic administration includes the suppression of the protein expression TGF- $\beta$ 1, p-Smad2 and circulating TGF- $\beta$ 1, but not p-p38. Our study added a new dimension to these results, by suggesting that chronic treatment with ANG-(1–7) may exert its effects on improving vascular remodeling after angioplasty in rabbits through the down-regulation of TGF- $\beta$ 1 and inhibition of the Smad2 pathway but not the TGF- $\beta$ /p38 pathway.

The continuous infusion of ANG-(1–7), however, only slightly decreased the circulating levels of TGF- $\beta$ 1 on the seventh day. In this respect, there was no significant difference between ANG-(1–7)-treated and saline-treated rabbits. This result may be reconciled with the findings from the study by Iwata et al. [9], in which pretreatment of ARCF with ANG-(1–7) slightly decreased the expression of TGF- $\beta$ 1 mRNA and did not have a significant inhibitory effect on ANGII-induced increases in TGF- $\beta$ 1 mRNA expression.

The enhanced expression and blood levels of TGF- $\beta$  have been observed in human with severe coronary heart diseases, advanced atherosclerosis and restenosis [20]. On the other hand, other studies have yielded conflicting results. Lutgens et al. [21] demonstrated that antagonism of TGF- $\beta$  increased plaque instability in animal models, which was in line with clinical observations [22] that low blood levels of active TGF- $\beta$  correlated with the severity of vascular disease. A variety of explanations for this inconsistency have been provided. The vast majority of investigators believed that TGF- $\beta$  exhibits different effects at different phases during the course of the disease [18]. During the early stage of injury, anti-TGF- $\beta$  enhanced cytokine and chemokine synthesis and increased VSMC proliferation and migration. In contrast, inhibition of TGF- $\beta$  during the later stages attenuated the deposition of ECM proteins and increased their degradation. Base upon our data, we surmise that ANG-(1–7) reduces vascular remodeling, possibly by regulating the expression of TGF- $\beta$ 1 in order to maintain the balance of TGF- $\beta$  in local vessel walls. The effects of ANG-(1–7) on the TGF- $\beta$  system were slow and durable.

An extensive body of scientific work [2] supports the concept that vascular remodeling after angioplasty predominantly depends upon VSMC proliferation and the increased expression of ECM proteins. Vascular fibrosis is caused by increased ECM deposition of total collagen by changes in the ratio of collagen type, fibronectin and proteoglycans and a reduction in the degradation of the ECM. Verrecchia et al. [23,24] reported that the TGF- $\beta$ /Smad signaling pathway has the potential to up-regulate the transcription of several genes that are important for ECM formation, such as the  $\alpha$ 1 and  $\alpha$ 2 chain of *Collagen I* and the  $\alpha$ 1 chain of *Collagen III*. Our measurements demonstrated that, when compared to the sham group, *Collagen I* was approximately 2.36-fold increased in the control group, 1.34-fold increased in the ANG-(1–7) group and 2.17-fold increased in the ANG-(1–7) + A-779 group. When compared to the sham group, *Collagen III* was 3.93-fold increased in the control group, 2.56-fold increased in the ANG-(1–7) group and 3.71-fold increased in the ANG-(1–7) + A-779 group. ANG-(1–7) can significantly reduce the mRNA expression of both *Collagen I* and *Collagen III* compared with the control group. There was no significant difference in the expression levels of *Collagen I* and *Collagen III* when the ANG-(1–7) + A-779 group was compared to the control group. These findings suggested that ANG-(1–7) reduced vascular remodeling and decreased collagen secretion and that these effects may possibly influence the TGF- $\beta$ /Smad signaling pathway.

## Conclusion

In summary, this study is the first to our knowledge that provides evidence that ANG-(1–7) reduces vascular remodeling, by

not only inhibiting neointimal formation but also decreasing collagen secretion. These effects are possibly mediated through the regulation of the TGF- $\beta$ /Smad signaling pathway in an in vivo, rabbit abdominal aorta injury model. These findings may lead to a new therapy for diseases that involve vascular remodeling in the presence or absence of injury.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.08.112.

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