Original Article
Ang (1-7) improves cardiac function after resuscitation from cardiac arrest

Xia Zhou[1,2*], Bin He[1*], Li Zhu[3*], Jia Huang[2], Hourong Zhou[2], Qisong Yuan[4], Yu Cao[1]

[1]West China Hospital of Sichuan University, Chengdu 610041, China; [2]Department of Emergency, Guizhou Provincial People's Hospital, Guiyang 550200, China; [3]The Second People's Hospital of Chengdu Xindu Distric, Chengdu 610500, China; [4]Guizhou Medical University, Guiyang 550200, China. *Equal contributors.

Abstract: Objective: The aim of this study was to investigate the mechanistic action and therapeutic effects of Ang (1-7) on myocardial dysfunction after cardiopulmonary resuscitation from cardiac arrest. Methods: Sprague-Dawley rats were subjected to tracheotomies, arteriovenous punctures, invasive mechanical ventilation, and cardiopulmonary resuscitation with angiotensin-(1-7) Ang (1-7) or without the peptide. The sham group of rats received tracheotomies, arteriovenous punctures, and cardiopulmonary resuscitation with saline. Heart rate (HR), mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and left ventricular maximal ascending and descending rates (± LVdp/dtmax) were measured before treatment (Baseline) and 6 hours after resuscitation. MicroRNA expression of Mas receptor (MasR), lncAng362, miR222, or phosphatase and tensin homolog (Pten) in myocardial tissues was analyzed by quantitative real-time PCR. Protein expression of MasR and Pten in myocardium was determined by Western blotting. Results: Ang (1-7) treatment significantly enhanced its receptor MasR and downstream molecule Pten expression and inhibited lncAng362 and miR222 expression in rat cardiac tissues after resuscitation from cardial arrest. Ang (1-7) treatment significantly improved rat cardiac function after resuscitation, with significant higher LVSP and LVDP, but lower LVEDP compared to control group. Conclusion: Ang (1-7) attenuated rat cardiac dysfunction after resuscitation from cardiac arrest by promoting MasR and Pten expression and suppressing lncAng362 and miR222 expression. Ang (1-7) could serve as a novel therapeutic agent for treatment of cardiac dysfunction after cardiac arrest.

Keywords: Cardiac insufficiency, cardiopulmonary resuscitation, Ang (1-7), lncAng362, Pten

Introduction

Cardiac arrest is a worldwide public health issue, with approximately 70-90% mortality and severe morbidity [1]. Patients surviving from cardiac arrest after cardiopulmonary resuscitation (CPR) mostly suffer from cardiac insufficiency, as well as other physical ailments [2]. Thus, attunating cardiac dysfunction after resuscitation is an effective measure improving the survival rate of patients with cardiac arrest.

Renin-angiotensin signaling pathways play an important role in cardiac insufficiency after resuscitation [3]. Triggering AT1R signaling by angiotensin II (AngII) suppresses CPR and activation of Mas receptor (MasR) signaling affects cardiac function after cardiac arrest [4]. Angiotensin-(1-7) Ang (1-7) is a key molecule in renin-angiotensin signaling pathways, mainly produced through the digestion of AngII by ACE2 [5]. Ang (1-7) has the ability to bind to Mas receptors (MasR) and function as a key vascular protective peptide [6, 7], inducing beneficial cardiovascular responses. However, it is still unclear whether Ang (1-7) promotes cardiac function after cardiopulmonary resuscitation from cardiac arrest.

lncAng362 is a long-chain non-coding RNA (lncRNA) which does not encode proteins but regulates epigenetic [8], transcription [9], and post-transcriptional gene expression [10]. lncAng362 is found near the site of microRNA miR-222 [11]. lncAng362 and miR-222 co-regulate the proliferation of blood vessels after the activation of AngII [11, 12]. miR-222 interacts with its target protein phosphatase and tensin homolog (Pten) and mediates multiple signaling pathways linked to a variety of cardiovascular
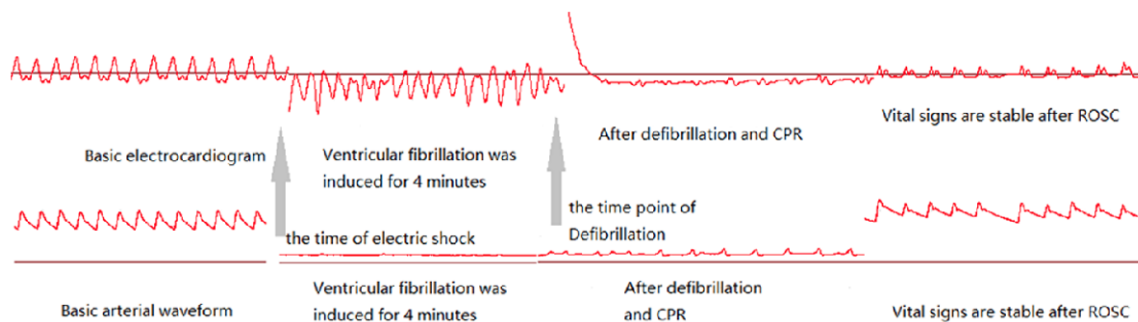
Figure 1. Experimental diagram. Mechanical ventilation of rats was performed for 30 minutes to ensure hemodynamic stability. Cardiac arrest was induced by ventricular fibrillation for 4 minutes. After cardiac arrest, ventilation was restored when chest compressions appeared. If necessary, a defibrillator was used at the same time. Return of spontaneous circulation (ROSC) was characterized once MAP continuous over 60 mmHg. When ROSC was restored, rats were divided into groups according to experimental design and monitored.

effects [13]. AngII can also generate Ang (1-7) under the action of angiotensin converting enzyme 2. However, the effects of Ang (1-7) on lncAng362 expression and consequent cardiac function are not fully understood. The present study investigated the function and mechanistic action of Ang (1-7) on myocardial dysfunction in a rat model of cardiopulmonary resuscitation after cardiac arrest.

Methods

Animals

Sprague-Dawley rats (8 weeks, 250-300 g) were randomly divided into three groups (n=10 per group): sham-operated group (Sham), cardiac arrest resuscitation group (Control), and resuscitation with Ang (1-7) treatment group (Ang (1-7)). The process of cardiac arrest resuscitation included CA, CPR, and return of spontaneous circulation (ROSC). Cardiac tissues were harvested from rats at 6 hours after ROSC. All rats used in the experiments were purchased from the Experimental Animal Center, Guiyang College of Traditional Chinese Medicine. Animal use was approved by the Animal Ethics Committee of Guizhou Provincial People's Hospital in China.

Cardiac arrest resuscitation and cardiac function monitoring

Rats were fasted with water for 12 hours before the operation, then subjected to anesthesia with intra-peritoneal injections of chloral hydrate (300-380 mg/kg), of which 100 mg/kg was administered every hour to maintain the effects. Transesophageal pacing induced ven-

tricular fibrillation rat cardiac arrest models were established, as described previously [14]. Briefly, mechanical ventilation was performed for 30 minutes to ensure hemodynamic stability in all the groups. The 5F pacing cardiac electrodes were inserted into the esophagus about 7 cm through the mouth. The proximal end of the pacemaker was connected to the output of the AC transformer. The 50 V AC 25 V stimuli was used to induce 90 s ventricular fibrillation. After stop stimulation, cardioversion changes were observed. When ventricular fibrillation automatically converted to sinus rhythm, the stimulation was immediately increased until the induction of ventricular fibrillation persisted [15] (**Figure 1**). Four minutes after ventricular fibrillation, electrical stimulation was stopped with immediate implementation of cardiopulmonary resuscitation. Chest compressions were performed with a modified Lanshike Pneumatic CPR machine (MCPR100A, Guangzhou Lanswick Co., Ltd., China), with a chest compressional frequency of 200 beats/minute and a depth of compression of 1/3. CPR began after the femoral vein epinephrine 0.02 mg/kg (administration volume of 2 ml/kg), up to 3 times 2-4J two-way defibrillation. ROSC standard was followed, as described previously [16]. During the experiment, rectal temperature was maintained in the normal range (37.8~38.7°C). Ventilation was assistanted with ALC-V9 animal ventilator (100% pure oxygen) (Shanghai Orte Biotech Co., Ltd., China), with the rate of 80 beats/min. The amount of gas was 6.5 mL/kg. BL-420S biological signal acquisition and analysis system (Chengdu Aosheng Technology Corporation, China) was used to measure cardiac function, such as heart rate (HR), mean arterial pressure (MAP), left
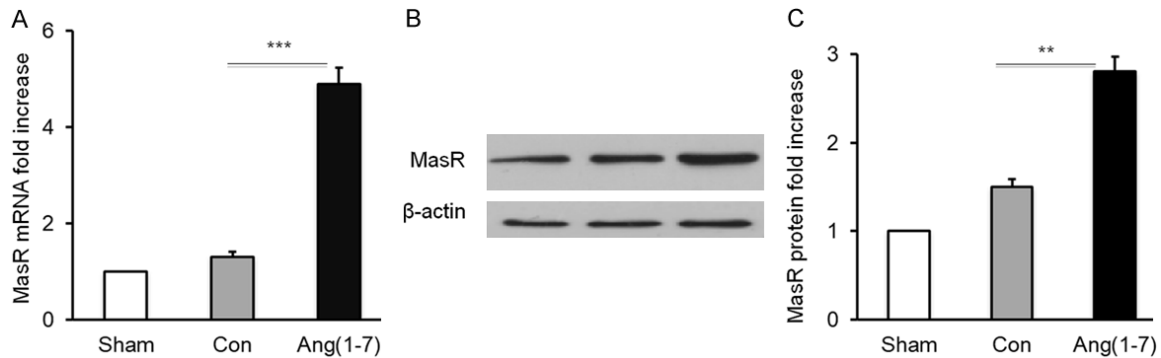
Figure 2. Effects of Ang (1-7) ligation on MasR expression in myocardial tissues. Myocardial tissues were isolated from rats in sham, Ang (1-7)-treated, or control group without Ang (1-7) treatment. Total RNA or protein was extracted from the tissues. A. RNA from the tissues of the three groups of rats was subjected to qRT-PCR. MasR mRNA expression fold increase in Ang (1-7)-treated or control group was calculated compared to the one in sham group. GAPDH gene served as internal control. B. MasR protein expression in myocardial tissues of the three groups of rats was examined by Western blot with anti-MasR specific antibodies and quantified with the BandScan 4.3 software. β-actin served as internal control. C. MasR protein expression levels in myocardial tissues from rats in control or Ang (1-7)-treated group were calculated as a fold increase in comparison with the sham group. Data are from three independent experiments, presented as mean ± SD and analyzed by One-way ANOVA. **$P<0.01$, ***$P<0.001$.

ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and left ventricular maximal ascending and descending rates (± LVdp/dtmax).

Administration of Ang (1-7)

Rats were intravenously administrated with Ang (1-7) (800 ng/kg/min) (MedChem Express Co., Ltd., Shanghai, China) when CPR began. Rats in the control group received intravenous injections of saline (2 ml/kg) saline.

Cardiac tissues

Rats were sacrificed by injections with a lethal dose of phenobarbital (250 mg/kg). Rat chests were opened and the hearts were isolated from the aortic root, rinsed with phosphate-buffered saline (PBS), and the left and right ventricles were separated. All cardiac tissues were immediately stored in a liquid nitrogen tank.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from cardiac tissues with TRIzol Reagent (Beijing Tiangen Biochemical Technology Co. Ltd., Beijing, China). cDNA was synthesized with PrimeScript RT reagent Kit (TaKaRa, Otsu, Japan). qRT-PCR was performed with SYBR Premix ExTaq II (TliRNaseH Plus), ROX plus (TaKaRa, Otsu, Japan), and primers under a PCR program: 95° for 30 sec-

onds followed by 45 cycles of 95°C for 5 seconds and 60°C for 40 seconds on an ABI 7500 fluorescence quantitative PCR system. Primers for IncAng362: forward 5'-TATGCGTGTTCCTG-CCTGTT-3', reverse 5'-ATCAAGAAATTAGACATT-CATGCCT-3'. Primers for MasR: forward 5'-AC-ATTATCAGTGACTTTTCTGTTTGG-3', reverse 5'-CAAGCAAGAAAGAGCCCACAG-3'. Primers for miR222: forward 5'-GCTACATCTGGCTACTGG-GT-3', reverse 5'-GGCCAACCGCGAGAAGATG-3'. Primers for Pten: forward 5'-TGAAGACCATAA-CCCACCACAGC-3', reverse 5'-CATTACACCAGTT-CGTCCCTTTCC-3'. Primers for GAPDH: forward 5'-GTCAGTGGTGGACCTGGACCT-3', reverse 5'-AGGGGAGATTCAGTGTGGTG-3'. Expression levels of MasR, Pten genes, IncAng362, and miR-222 were analyzed with $2^{-\Delta\Delta CT}$ method. GAPDH gene served as the internal control.

Western blot

Proteins were extracted from myocardial tissue with RIPA lysis buffer, according to manufacturer instructions (Beyotime Institute of Biotechnology, Haimen, China). Protein concentrations were determined with the BCA assay (Sigma, St Louis, MO, USA). Proteins (50 μg/sample) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto polyvinylidenedifluoride membranes (Millipore corp., Billerica, MA, USA). The membranes were blocked with 5% non-fat milk in TBST (10 mM Tris, pH 7.4,
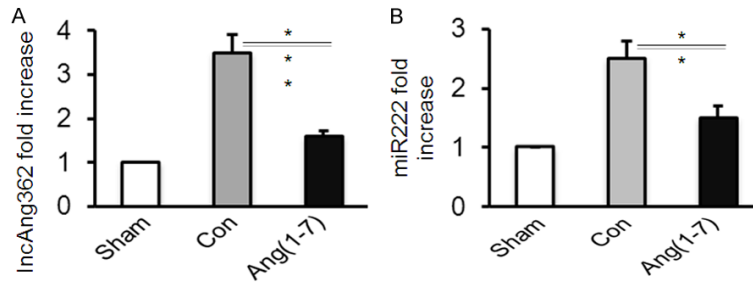
Figure 3. Effects of Ang (1-7) stimulation on lncAng362 and miR222 expression in myocardial tissues. Total RNA was extracted from rat myocardial tissues in sham, Ang (1-7)-stimulated, or control group and subjected to qRT-PCR. lncAng362 (A) or miR222 (B) expression fold change in Ang (1-7)-treated or control group was calculated in comparison with the sham group. GAPDH gene served as internal control. Data are from three independent experiments, presented as mean ± SD, and analyzed by One-way ANOVA. **P<0.01, ***P<0.001.

(**Figure 2A**). This study further profiled MasR protein expression in myocardial tissues of the three groups of rats. Western blotting showed that Ang (1-7) treatment markedly enhanced MasR protein production in the tissues, compared to sham or control treatment without Ang (1-7) (**Figures 2B**, S1), with a 4.9 fold increase on average (**Figure 2C**).

Ang (1-7) treatment suppressed lncAng362 and miR222 production in myocardial tissues

150 mM NaCl, and 0.1% Tween-20) at room temperature for 1 hour and reacted with anti-rat Mas or Pten primary antibodies (1:200) or anti-b-actin antibody (1:1000) overnight at 4°C, followed with the secondary antibody (HRP, 1:5000) for 2 hours at room temperature. Membranes were developed with ECL chemiluminescence. Protein expression was analyzed with BandScan 4.3 software.

Statistical analysis

Data are presented as mean ± mean ± SD (standard deviation) and were analyzed by One-way ANOVA, using SPSS 19.0 software (IBM, Armonk, NY, USA). Bivariate correlation was used in correlation analysis and Pearson's correlation coefficient was used in two-tailed tests. *P* < 0.05 indicates statistical significance.

Results

Ang (1-7) ligation enhanced MasR expression in myocardial tissues

MasR plays a key role in cardiac remodeling and fibrosis in post-myocardial infarction [17]. To examine the effects of Ang (1-7) ligation on MasR expression after resuscitation from cardiac arrest, this study established rat ventricular fibrillation cardiac arrest models by using transesophageal pacing and treated the rats with Ang (1-7). Quantification of MasR mRNA expression in myocardial tissues by qRT-PCR demonstrated that Ang (1-7) significantly promoted MasR gene expression compared with the one without Ang (1-7) or sham treatment

lncAng362 and miR222 have been associated with AngII-induced vascular smooth muscle cell proliferation [11] and blood vessels. To test the effects of Ang (1-7) ligation on expression of lncAng362 and miR222, this study quantified transcripts of both RNAs in myocardial tissues in rats resuscitated from cardial arrest by qRT-PCR. Analyses of gene mRNA in the tissues demonstrated that Ang (1-7) significantly reduced expression levels of both lncAng362 (**Figure 3A**) and miR222 (**Figure 3B**), compared with controls absent from Ang (1-7).

Ang (1-7) promoted Pten expression in myocardial tissues

Pten is essential for the control of vascular density and vessel growth in a cell-autonomous and dose-dependent manner [18]. To test whether Ang (1-7) treatment could affect Pten gene expression, this study analyzed its mRNA and protein production in myocardial tissues in rats resuscitated from cardial arrest. qRT-PCR demonstrated that Ang (1-7) treatment significantly promoted Pten mRNA expression in the tissues, with more than a 3-fold increase compared with the controls without Ang (1-7) (**Figure 4A**). Western blotting further confirmed that Ang (1-7) markedly enhanced Pten protein production in the tissues (**Figures 4B** and **4C**, S2).

Ang (1-7) treatment improved cardiac function after arrest resuscitation

To examine the effects of Ang (1-7) treatment on cardiac function after resuscitation from cardiac arrest, this study measured the param-
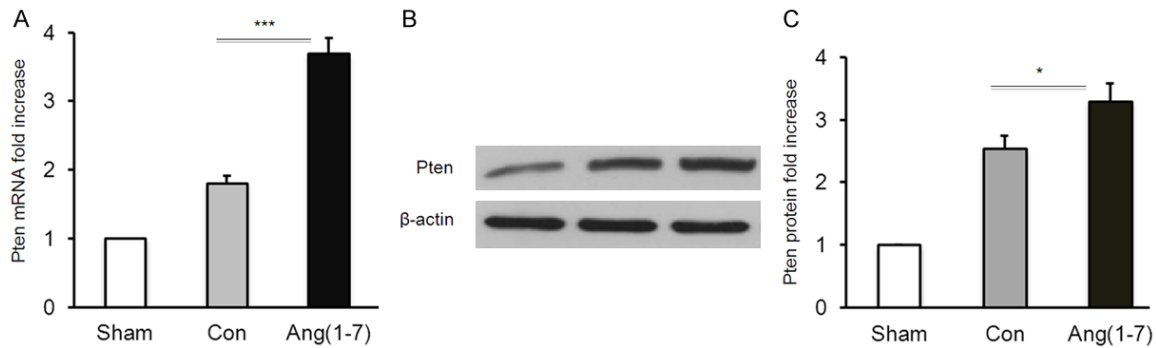
Figure 4. Figure 4. Effects of Ang (1-7) treatment on Pten expression in myocardial tissues. Myocardial tissues were isolated from rats in sham, Ang (1-7)-treated, or control group. A. Total RNA was extracted from myocardial tissues of rats in the three groups and subjected to qRT-PCR. Pten mRNA expression fold increase was calculated compared to the sham group. GAPDH gene served as internal control. B. Pten protein expression in rat myocardial tissues was examined by Western blot with anti-Pten antibodies. β-actin served as internal control. C. Pten protein expression was quantified with the BandScan4.3 software. Pten protein expression fold increase in Ang (1-7)-treated or control group was calculated in comparison with the one in the sham group. Data are from three independent experiments, presented as mean ± SD, and analyzed by One-way ANOVA. *$P<0.05$, ***$P<0.001$.

eters of cardiac function. Parameters of cardiac performance, such as LVSP, ±LVdp/dtmax, LVEDP, MAP, and HR were observed in rats before and after treatment. No significant differences in baseline (before surgery) parameters such as total body weight, chloral hydrate, CPR, HR, MAP, LVSP, LVEDP, +LVdp/dtmax and -LVdp/dtmax were observed among the three groups of rats. Measurement of those parameters after the operation demonstrated that rats in the control group under resuscitation from cardiac arrest had lower LVSP (**Figure 5A**) and LVdp/dtmax (**Figure 5C** and **5D**), but higher LVEDP (**Figure 5B**), compared to the sham group. In contrast, rats receiving Ang (1-7) treatment after resuscitation had significantly higher LVSP (**Figure 5A**), lower LVEDP (**Figure 5B**), and higher + LVdp/dtmax (**Figure 5C**) and -LVdp/dtmax (**Figure 5D**), compared to the control group without Ang (1-7) treatment (**Figure 5A-D**). No significant differences were observed in MAP or HR in rats between the Ang (1-7)-treated group and control group (Data not shown). This study further analyzed the relationship between expression of lncAng362 and cardiac function in myocardial tissue. Correlation analyses demonstrated that lncAng362 expression in myocardial tissues had significant negative correlation with LVSP (**Figure 6A**) and LVdp/dtmax (**Figure 6B** and **6C**), but had significant positive correlation with LVEDP (**Figure 6D**).

Discussion

The present study demonstrated that Ang (1-7) ligation significantly enhances expression of its receptor MasR, inhibits expression of lncAng362 and miR222, increases Pten molecule production in rat myocardial tissues, and consequently improves rat cardiac function after resuscitation *in vivo*. To the best of our knowledge, this is the first report showing that Ang (1-7) has the ability to enhance cardiac performance after cardiopulmonary resuscitation from cardiac arrest.

Ang (1-7) has been shown to induce expression of vascular endothelial growth factor and matrix metalloproteinase-9 [19] and improve vascular endothelial function, delaying the development of heart failure after myocardial infarction [20]. Absence of Ang (1-7)/MasR ligation leads to heart dysfunction *in vivo*, decreasing left ventricular filling pressure, LVdp/dtmax, cardiac output, and cardiac index in Mas deficiency [21]. The present study found that Ang (1-7) treatment after resuscitation from cardiac arrest markedly enhanced expression of MasR in myocardium, suggesting a novel molecular mechanism by which Ang (1-7) exhibits protective effects on the cardiovascular system.

lncAng362 and miR222 are two types of noncoding RNAs linked with the Ang (1-7)/MasR signaling complex. It has been shown that Ang (1-7) downregulated the expression of lncAng362 and miR222. Moreover, specific knockdown of lncAng362 inhibited miR222 expression [11], indicating that lncAng362 collaborates with miR222 in response to upstream Ang (1-7) signaling. This study also showed that ligation of Ang (1-7) with MasR markedly
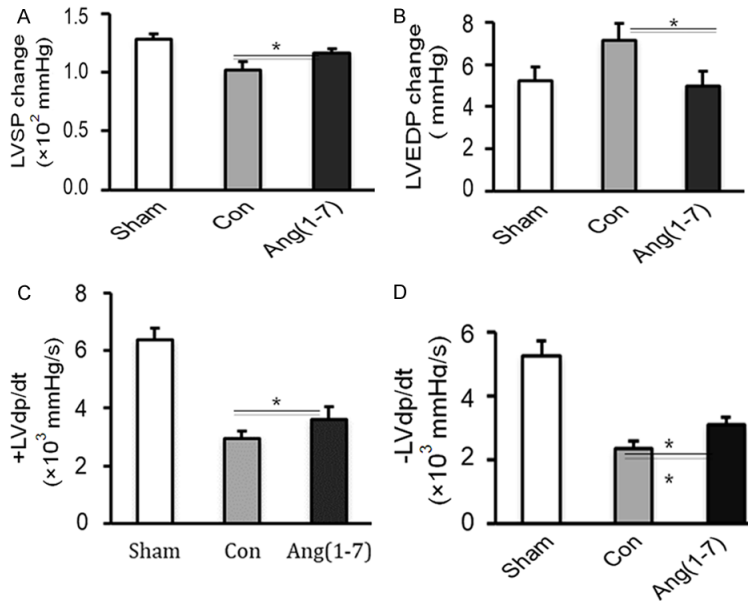
Figure 5. Effects of Ang (1-7) treatment on cardiac function after cardiopulmonary resuscitation from cardial arrest. Rats were divided into three groups: sham group, Ang (1-7), or saline (Con) intravenous administattion group after cardiopulmonary resuscitation. Parameters of cardiac function LVSP (A), LVEDP (B), +LVdp/dt (C) or -LVdp/dt (D) were monitored with BL-420S biological signal acquisition and analysis system. Data are from three independent experiments, presented as mean ± SD, and analyzed by one-way ANOVA. *$P < 0.05$, **$P < 0.01$.
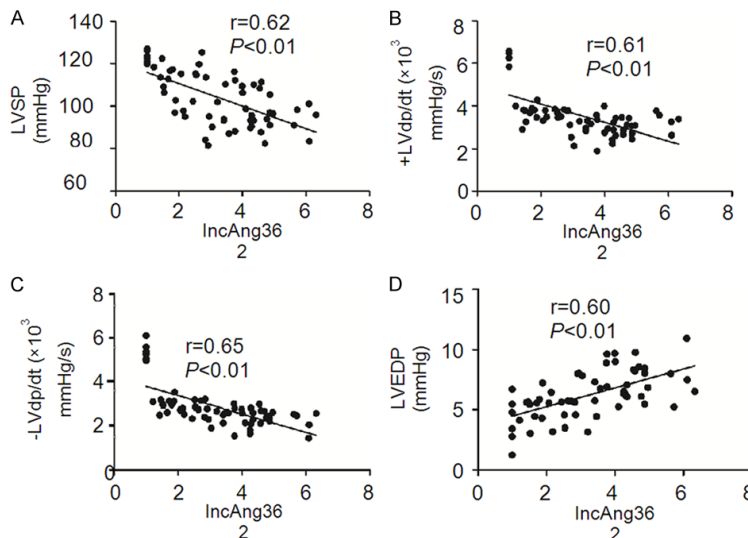

Figure 6. Correlation of lncAng362 and parameters of cardiac function. Bivariate correlation analysis between lncAng362 and LVSP (A), +LVdp/dt (B), or -LVdp/dt (C), or LVEDP (D) was performed using One-way ANOVA. Pearson's correlation coefficient was used in two-tailed tests. $P < 0.05$ is considered to be statistically significant.

relation between lncAng362 and miR222 and a possible co-regulation relationship between lncAng362 and miR222 expression in myocardial tissues after cardiopulmonary resuscitation. However, different from previous reports based on vascular smooth muscle cell culture system *in vitro* [12], it was found that increased expression of lncAng362 in the control group of rats aggravated myocardial injuries after resuscitation from cardiac arrest *in vivo*.

Pten is widely expressed in the cardiovascular system and plays an important role in cardiovascular disease [22]. Numerous studies have found that overexpression of Pten negatively regulates cardiomyocyte hypertrophy induced by AngII through multiple pathways [23, 24]. MicroRNAs bind to the 3'untranslated region of target messenger RNA, inhibiting their translation or directly degrading them and regulating the post-transcriptional level of genes, thereby regulating cell proliferation, differentiation, and apoptosis [25]. Pten is a direct target of miR222 that can regulate the expression of Pten at a post-transcriptional level [26]. It has been shown that Pten can inhibit the activation of AKT/eNOS signaling pathways and protect the heart [27]. In a cardiac fibrosis model, Pten overexpression can inhibit collagen metabolism and matrix metalloproteinases synthesis, thus inhibiting cardiac fibrosis [26]. The present study showed that Ang (1-7) significantly increased expression of Pten in rat myocardial tissues after cardiopulmonary resuscitation from cardiac arrest. It is possible that the mechanistic action

reduced expression of both lncAng362 and miR222, suggesting that there is a positive correlation
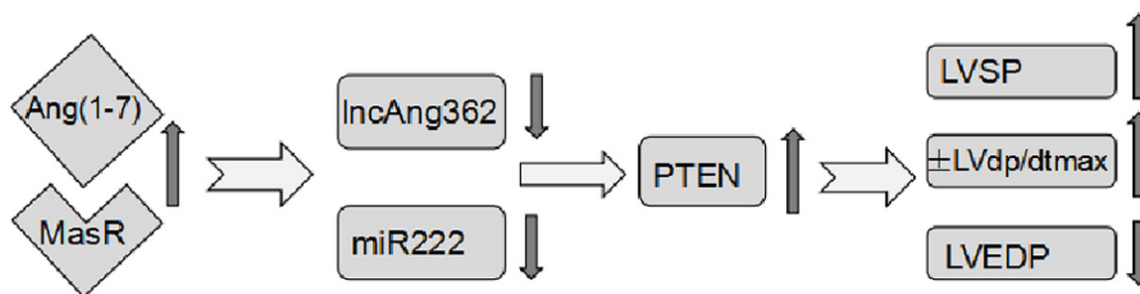
Figure 7. Proposed mechanistic action of Ang (1-7) on rat cardiac function. MasR gene expression in myocardial tissues was enhanced by Ang (1-7) ligation, which lead to the downregulation of lncAng362 and miR222 expression and consequent upregulation of Pten gene expression, resulting in the increase of rat LVSP and LVdp/dtmax, decrease of LVEDP, and improvement of rat cardiac function after cardiopulmonary resuscitation from cardiac arrest.

of lncAng362 on cardiac function might be through the regulation of miR222 and Pten expression in myocardial tissues. Based on results, it is suggested that the mechanistic action of Ang (1-7) on cardiac function after cardiopulmonary resuscitation might be through the upregulation of MasR, downregulation of lncAng362 and miR222, and upregulation of Pten expression in myocardial tissues (**Figure 7**).

In conclusion, Ang (1-7) treatment attenuates cardiac insufficiency after cardiopulmonary resuscitation from cardiac arrest. The present study suggests that Ang (1-7) could serve as a potent therapeutic reagent for treatment of myocardial dysfunction after resuscitation.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yu Cao, West China Hospital of Sichuan University, Chengdu 610041, China. Tel: 86-28-8542-2288; E-mail: dr.yu.cao@gmail.com; Dr. Hourong Zhou, Department of Emergency, Guizhou Provincial People's Hospital, Guiyang 550200, China. Tel: 86-139-851-36428; E-mail: zhr1974@163.com

References

[1] Harris AW and Kudenchuk PJ. Cardiopulmonary resuscitation: the science behind the hands. Heart 2018; 104: 1056-1061.

[2] Bougouin W and Cariou A. Cardiac issues in cardiac arrest. Semi Neurol 2017; 37: 13-18.

[3] Mollace V, Gliozzi M, Capuano A and Rossi F. Modulation of RAAS-natriuretic peptides in the treatment of HF: old guys and newcomers. Int J Cardiol 2017; 226: 126-131.

[4] Wang G, Zhang Q, Yuan W, Wu J and Li C. Sildenafil protects against myocardial ischemia-reperfusion injury following cardiac arrest in a porcine model: possible role of the renin-angiotensin system. Int J Mol Sci 2015; 16: 27015-27031.

[5] Santos RAS, Sampaio WO, Alzamora AC, Motta-Santos D, Alenina N, Bader M and Campagnole-Santos MJ. The ACE2/Angiotensin-(1-7)/MAS axis of the renin-angiotensin system: focus on angiotensin-(1-7). Physiol Rev 2018; 98: 505-553.

[6] Santos RA, Ferreira AJ, Verano-Braga T and Bader M. Angiotensin-converting enzyme 2, angiotensin-(1-7) and Mas: new players of the renin-angiotensin system. J Endoc 2013; 216: R1-R17.

[7] Patel VB, Zhong JC, Grant MB and Oudit GY. Role of the ACE2/angiotensin 1-7 axis of the renin-angiotensin system in heart failure. Cir Res 2016; 118: 1313-1326.

[8] Ntziachristos P, Abdel-Wahab O and Aifantis I. Emerging concepts of epigenetic dysregulation in hematological malignancies. Nat Immunol 2016; 17: 1016-1024.

[9] Yu Y, Yarrington RM, Chuong EB, Elde NC and Stillman DJ. Disruption of promoter memory by synthesis of a long noncoding RNA. PNAS 2016; 113: 9575-9580.

[10] Yoon JH, Abdelmohsen K and Gorospe M. Posttranscriptional gene regulation by long noncoding RNA. J Mol Biol 2013; 425: 3723-3730.

[11] Leung A, Trac C, Jin W, Lanting L, Akbany A, Saetrom P, Schones DE and Natarajan R. Novel long noncoding RNAs are regulated by angiotensin II in vascular smooth muscle cells. Cir Res 2013; 113: 266-278.

[12] Leung A, Stapleton K and Natarajan R. Functional long non-coding RNAs in vascular smooth muscle cells. Cur Top Microbiol Immunol 2016; 394: 127-141.

[13] Mishra PK, Tyagi N, Kumar M and Tyagi SC. MicroRNAs as a therapeutic target for cardiovascular diseases. J Cell Mol Med 2009; 13: 778-789.

[14] Chen MH, Liu TW, Xie L, Song FQ, He T, Zeng ZY and Mo SR. Ventricular fibrillation induced by transoesophageal cardiac pacing: a new model of cardiac arrest in rats. Resuscitation 2007; 74: 546-551.

[15] Martins HS, Koike MK and Velasco IT. Effects of terlipressin and naloxone compared with epinephrine in a rat model of asphyxia-induced cardiac arrest. Clinics 2013; 68: 1146-1151.

[16] Wang P, Yao L, Zhou LL, Liu YS, Chen MD, Wu HD, Chang RM, Li Y, Zhou MG, Fang XS, Yu T, Jiang LY and Huang ZT. Carbon monoxide improves neurologic outcomes by mitochondrial biogenesis after global cerebral ischemia induced by cardiac arrest in rats. Int J Biol Sci 2016; 12: 1000-1009.

[17] Wang J, He W, Guo L, Zhang Y, Li H, Han S and Shen D. The ACE2-Ang (1-7)-Mas receptor axis attenuates cardiac remodeling and fibrosis in post-myocardial infarction. Mol Med Reports 2017; 16: 1973-1981.

[18] Serra H, Chivite I, Angulo-Urarte A, Soler A, Sutherland JD, Arruabarrena-Aristorena A, Ragab A, Lim R, Malumbres M, Fruttiger M, Potente M, Serrano M, Fabra À, Viñals F, Casanovas O, Pandolfi PP, Bigas A, Carracedo A, Gerhardt H and Graupera M. PTEN mediates Notch-dependent stalk cell arrest in angiogenesis. Nat Commun 2015; 6: 7935.

[19] Zhao W, Zhao T, Chen Y and Sun Y. Angiotensin 1-7 promotes cardiac angiogenesis following infarction. Cur Vascul Pharmacol 2015; 13: 37-42.

[20] Durik M, van Veghel R, Kuipers A, Rink R, Haas Jimoh Akanbi M, Moll G, Danser AH and Roks AJ. The effect of the thioether-bridged, stabilized Angiotensin-(1-7) analogue cyclic ang-(1-7) on cardiac remodeling and endothelial function in rats with myocardial infarction. Int J Hypert 2012; 2012: 536426.

[21] Botelho-Santos GA, Bader M, Alenina N and Santos RA. Altered regional blood flow distribution in Mas-deficient mice. Therap Adv Cardiovascul Disease 2012; 6: 201-211.

[22] Breton-Romero R, Kalwa H, Lamas S and Michel T. Role of PTEN in modulation of ADP-dependent signaling pathways in vascular endothelial cells. Biochim Biophys Acta 2013; 1833: 2586-2595.

[23] Ye S, Yang L, Zhao X, Song W, Wang W and Zheng S. Bioinformatics method to predict two regulation mechanism: TF-miRNA-mRNA and lncRNA-miRNA-mRNA in pancreatic cancer. Cell Biochem Biophys 2014; 70: 1849-1858.

[24] Calderaro J, Rebouissou S, de Koning L, Masmoudi A, Herault A, Dubois T, Maille P, Soyeux P, Sibony M, de la Taille A, Vordos D, Lebret T, Radvanyi F and Allory Y. PI3K/AKT pathway activation in bladder carcinogenesis. Int J Cancer 2014; 134: 1776-1784.

[25] Oudit GY, Kassiri Z, Zhou J, Liu QC, Liu PP, Backx PH, Dawood F, Crackower MA, Scholey JW and Penninger JM. Loss of PTEN attenuates the development of pathological hypertrophy and heart failure in response to biomechanical stress. Cardiovascul Res 2008; 78: 505-514.

[26] Nie L, Zhao JH, Wang J, Song R and Zhu SJ. Effect of phosphatase and tensin homologue on chromosome 10 on angiotensin II-mediated proliferation, collagen synthesis, and Akt/P27 signaling in neonatal rat cardiac fibroblasts. BioMed Res Int 2016; 2016: 2860516.

[27] Mocanu MM and Yellon DM. PTEN, the Achilles' heel of myocardial ischaemia/reperfusion injury? Br J Pharmacol 2007; 150: 833-838.
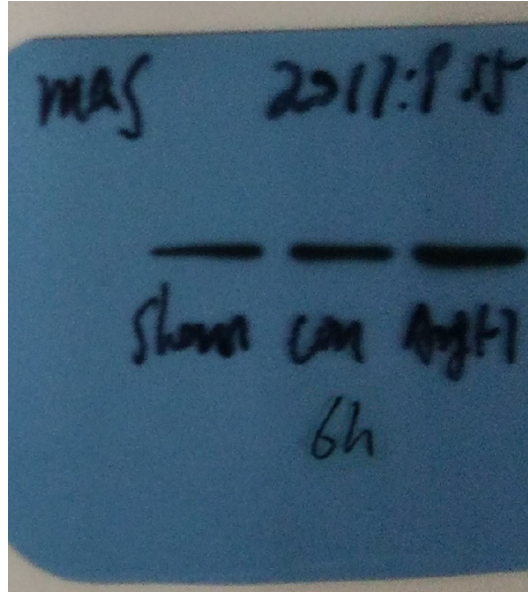
Western Blot, MasR

Figure S1. Supplemental whole film for **Figure 2B**. Total proteins were extracted from myocardial tissues of the three groups of rats (Sham, control or Ang (1-7) treatment), and subjected to gel electrophoresis. MasR protein expression in the tissues was examined by Western blot with anti-MasR specific antibodies.
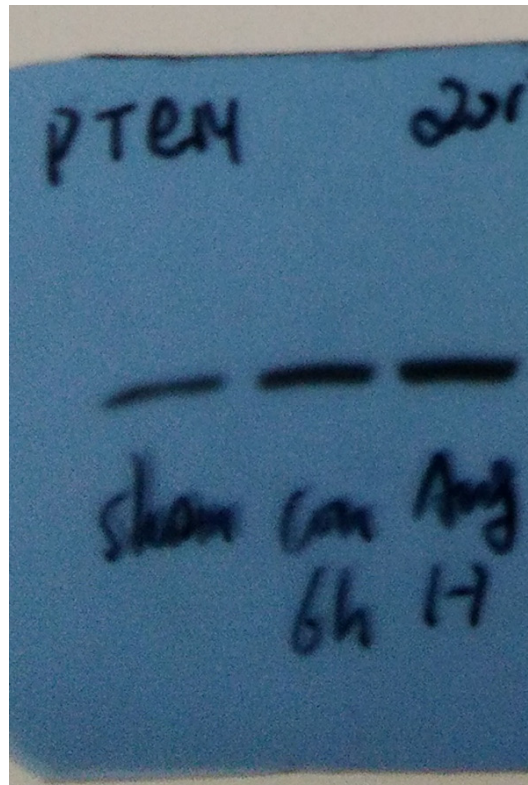
Western Blot, Pten

Figure S2. Supplemental whole film for **Figure 4B**. Total proteins were extracted from myocardial tissues of rats in Sham, control or Ang (1-7)-treated group. Pten protein expression in the tissues was examined by Western blot with anti-Pten specific antibodies.