



# Angiotensin-(1–7) attenuates collagen-induced arthritis via inhibiting oxidative stress in rats

Juan Liu<sup>1</sup> · Yan Liu<sup>1</sup> · Wenyu Pan<sup>1</sup> · Yongsheng Li<sup>1</sup>

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## Abstract

The present study was designed to investigate the anti-rheumatic effects and the mechanism of angiotensin (Ang)-(1–7) in rat models with collagen-induced arthritis (CIA). The CIA model was established using male Wistar rats by intradermal injection of bovine collagen-II in complete Freund's adjuvant at the base of the tail. The levels of angiotensin converting enzyme 2 (ACE2)/Ang-(1–7)/Mas receptor (MasR) were reduced in CIA rats. The attenuation of paw swelling and arthritis scores and improvement of indexes of spleen and thymus were done by Ang-(1–7) injection in CIA rats. The increased levels of inflammatory cytokines, such as interleukin (IL)-1 $\beta$ , IL-6, tumor necrosis factor (TNF)- $\alpha$ , and interferon (IFN)- $\gamma$  in the serum and hind paw were blocked by Ang-(1–7) administration. In addition, enhanced NADPH oxidase (Nox) activity, increased levels of superoxide anions and malondialdehyde (MDA), and weakened superoxide dismutase (SOD) activity, were all reversed by treatment with Ang-(1–7). Nox1 overexpression reversed the suppressing effects of Ang-(1–7) on paw swelling and arthritis scores in CIA rats. The Ang-(1–7)-induced improvement in spleen and thymus indexes in CIA rats was abolished by Nox1 overexpression. Nox1 overexpression reversed the inhibitory effects of Ang-(1–7) by increasing IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and IFN- $\gamma$  levels in the serum and hind paw of CIA rats. These results demonstrated that Nox1 increased the oxidative stress in arthritis, and Ang-(1–7) improved rheumatism in arthritis via inhibiting oxidative stress.

**Keywords** Collagen-induced arthritis · Rat · Rheumatism · Inflammatory cytokines · Oxidative stress

## Abbreviations

ACE	Angiotensin-converting enzyme
AIA	Adjuvant-induced arthritis
Ang	Angiotensin
AT1R	Angiotensin type 1 receptor
CIA	Collagen-induced arthritis
IFN	Interferon
IL	Interleukin
MasR	Mas receptor
MDA	Malondialdehyde
MLU	Mean light units
Nox	NADPH oxidase
RA	Rheumatoid arthritis
RAS	Renin–angiotensin system

ROS	Reactive oxygen species
TNF	Tumor necrosis factor

## Introduction

Rheumatoid arthritis (RA) is an autoimmune and chronic inflammatory disease characterized by joint tenderness, joint swelling and destruction of synovial joints, leading to severe disability (Jia et al. 2016; Croia et al. 2019; Sparks 2019). It is influenced by both genetic and environmental factors, showing a significant impact on occupational and daily activities, as well as mortality (Harris 1990; Scott et al. 2010; Deane et al. 2017). RA is usually polyarticular and typically presents with stiffness and pain in multiple joints, most often the metacarpophalangeal joints, proximal interphalangeal joints and wrist (Mouterde et al. 2019; De Cock et al. 2019). Therefore, it is imperative and urgent for clinicians and pharmacologists to develop safer, effective and economical drugs to attenuate joint pain and inflammation, as well as improve the quality of life of RA patients.

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✉ Yongsheng Li  
prettyhurricane@163.com

<sup>1</sup> Department of Rheumatology, The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, 1 Huanghe West Road, Huai'an 223300, China

The renin–angiotensin system (RAS) is a hormonal system that is traditionally known to regulate the effects of blood pressure. However, there is an increasing evidence that RAS signaling plays an important role in the inflammatory response associated with several diseases such as arthritis (Ranjbar et al. 2019). Several studies have shown that the major components of RAS including angiotensin II type 1 receptor (AT1R), angiotensin converting enzyme (ACE) and AT2R are expressed in the synovial tissues of animals as well as humans during the pathogenesis of RA and osteoarthritis. The expression levels of these are associated with the degree of inflammation and the severity of arthritis (Wang et al. 2018; Liu et al. 2016; Mackenzie et al. 2013). Losartan, an antagonist of AT1R, can be used as an effective RA treatment and exhibits anti-arthritic effects (Berk et al. 2007).

The discovery of ACE2/angiotensin (Ang)-(1–7)/Mas receptor (MasR) over the last decades has added a new dimension to the RAS family. Ang-(1–7) is an important biologically active peptide in the RAS family that is involved in regulating inflammation (Lelis et al. 2019; Simoes e Silva et al. 2013). Administration of MasR agonist AVE 0991 or Ang-(1–7) decreased hypernociception, neutrophil accumulation and production of inflammatory cytokines in adjuvant-induced arthritis (AIA) mice (da Silveira et al. 2010). Ang-(1–7) is a mediator of resolution of inflammation in antigen-induced arthritis mice (Barroso et al. 2017). However, whether Ang-(1–7) can improve RA is not well known.

Reactive oxygen species (ROS) is the most important class of radicals generated in living systems, and play an important role in cell signaling, proliferation, apoptosis, growth and differentiation under physiological conditions (Bellezza et al. 2020; Agidigbi and Kim 2019; Ebrahimi et al. 2020). However, the concentrations of ROS are increased beyond physiological conditions or imbalances between oxidants and antioxidants can cause damage to the cellular components (Dan Dunn et al. 2015) and inflammatory responses (Gao et al. 2020). However, whether Ang-(1–7) attenuates arthritis via restraining oxidative stress is not well understood. Therefore, the present study aimed to explore whether Ang-(1–7) could attenuate collagen-induced arthritis (CIA) in rats via inhibiting oxidative stress.

## Materials and methods

### Animals

Male Wistar rats (Vital River Biological Co., Ltd, Beijing, China) weighing 160–180 g (5–6 weeks) were used for experiments. All the rats should be approved by the Animal Ethics Committee of Nanjing Medical University and conformed to the National Institute of Health guidelines on the ethical use of animals for conducting research. The

ethical approval number was 17062846 and the study was approved in June 2017. Animals were housed under a 12 h light/dark cycle and fed with a standard chow diet and water.

### CIA and treatment

Collagen type II (Sigma, MO, USA) was emulsified in complete Freund's adjuvant (1 mg/ml, Sigma, MO, USA), and then 200  $\mu$ l collagen type II emulsion was intradermally injected at the base of the rat tail. After 7 days, 100  $\mu$ l booster immunization was done at the base of the rat tail, avoiding injection to the primary site. The first immunization was recorded as day 0. At the same time, control rats were treated with saline or Ang-(1–7) (Sigma, 300  $\mu$ g/kg/day) by intraperitoneal (i.p.) injection daily without collagen II in Freund's adjuvant.

### Experimental design

Experiment 1: The rats were randomly divided into four groups: control, Ang-(1–7), collagen-induced arthritis (CIA), and CIA + Ang-(1–7) groups, with  $N=8$  in the control and Ang-(1–7) groups, and  $N=12$  in the CIA and CIA + Ang-(1–7) groups. The rats were observed daily for clinical signs of arthritis. After 28 days, the spleen and thymus indexes, levels of ACE2/Ang-(1–7)/MasR, inflammatory cytokines and oxidative stress were determined.

Experiment 2: The rats were randomly divided into five groups: control, CIA, CIA + Ang-(1–7), CIA + Ad-Nox1 and CIA + Ang-(1–7) + Ad-Nox1 groups, with  $N=8$  in the control group, and  $N=12$  in the CIA, CIA + Ang-(1–7), CIA + Ad-Nox1 and CIA + Ang-(1–7) + Ad-Nox1 groups. The rats were observed daily for clinical signs of arthritis. After 28 days, the spleen and thymus indexes, and levels of inflammatory cytokines were determined.

### Arthritis assessment

All the rats were observed daily for clinical signs of arthritis. The paws were examined, and the severity and the swelling loci were graded on a 5-point scale: 0 = no swelling or erythema, 1 = slight swelling and/or erythema, 2 = low-to-moderate edema, 3 = pronounced edema with limited joint usage, and 4 = excess edema with joint rigidity. The total score for each rat was calculated as an arthritis index, with a maximum value of 8 (4 points  $\times$  2). The volume of the hind paw was determined using a paw volume plethysmometer.

### Assay of spleen and thymus indexes

On day 28 after immunization, the rats were sacrificed using an overdose of pentobarbital (100 mg/kg, i.p.). The spleen and the thymus were promptly removed and weighted. The spleen

and the thymus indexes were defined as the ratio of spleen or thymus wet weight versus the body weight (mg/g).

### Determination of Ang-(1-7)

The hind paw samples were homogenized in lysis buffer (Thermo Fisher Scientific, MA, USA). The total protein in the homogenate was extracted and measured using a BCA protein assay kit (BioChannel Biotechnology Co., Ltd., Nanjing, China). Ang-(1-7) was determined using ELISA kits (USCN Business Co., Ltd., Wuhan, China) according to the manufacturer's instructions.

### Western blotting

The hind paw samples were sonicated in lysis buffer (Thermo Fisher Scientific, MA, USA) and homogenized. The debris was removed by centrifugation at  $12,000\times g$  for 10 min at  $4\text{ }^{\circ}\text{C}$  and then the supernatant was collected. Subsequently, 30–40  $\mu\text{g}$  protein was separated by gel electrophoresis, and transferred onto PVDF membranes. The membrane was blocked with 5% skimmed milk powder at room temperature for 1 h and probed with primary antibodies for overnight at  $4\text{ }^{\circ}\text{C}$  against ACE2 (Abcam, MA, USA), MasR (Alomone Labs, Israel), IL-1 $\beta$  (Abcam), IL-6 (Abcam), TNF- $\alpha$  (Abcam), IFN- $\gamma$  (Abcam) and Nox1 (Abcam). Horseradish peroxidase-conjugated secondary antibody (Abcam) was added and incubated at room temperature for 1 h and GAPDH (Abcam) was used as an internal control. The bands were visualized via ECL

(Beyotime, Shanghai, China), and the images were analyzed using Image-Pro Plus software.

### Determination of serum levels of inflammatory cytokines

The serum samples of the rats were obtained and frozen at  $-80\text{ }^{\circ}\text{C}$  until use. The levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and IFN- $\gamma$  in the serum were determined by ELISA kits (USCN Business Co., Ltd.) following the manufacturer's instructions.

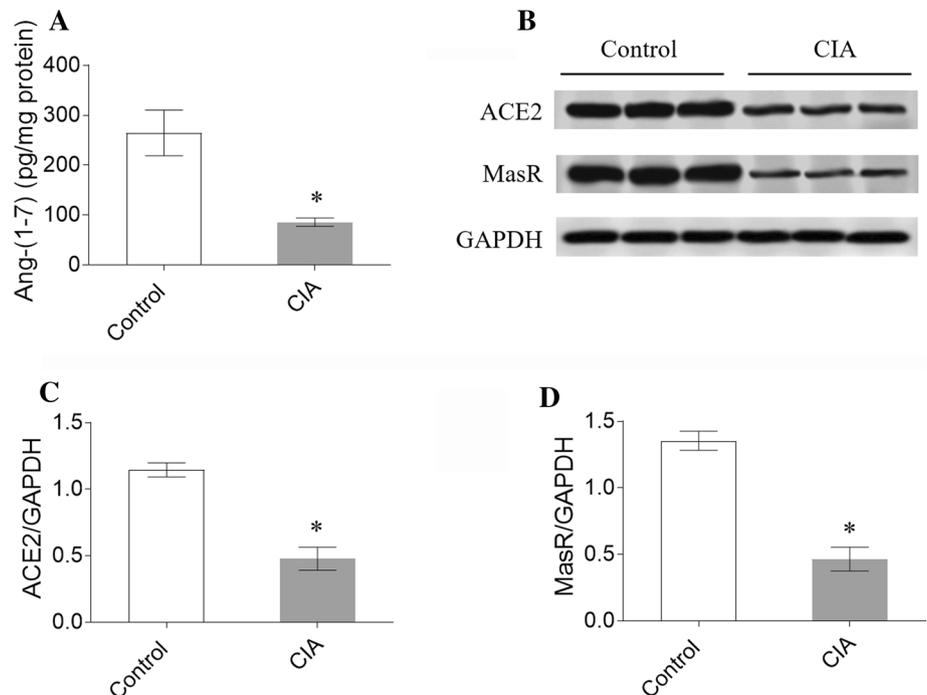
### Nox1 overexpression

Recombinant adenoviral vectors harboring Nox (Ad-Nox1) or green fluorescent protein (Ad-GFP) were obtained from Genechem Company Ltd. (Shanghai, China). Adenovirus ( $1\times 10^{10}$  TU/ml) was injected into the tail vein of the rats on days 0 and 14.

### Determination of superoxide dismutase (SOD) activity

On day 28, the hind paw samples were obtained and homogenized in lysis buffer (Thermo Fisher Scientific, MA, USA). SOD in these samples was measured using a microplate reader (BioTek, VT, USA) according to the manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, China).

**Fig. 1** Expression of angiotensin converting enzyme 2 (ACE2)/angiotensin (Ang)-(1-7)/Mas receptor (MasR). The levels of Ang-(1-7) in the serum, and ACE2 and MasR in the hind paw of collagen-induced arthritis (CIA) rats was lower than that in the control rats. The results are expressed as means  $\pm$  SE.  $N=8$  in the control group, and  $N=12$  in the CIA group.  $*P<0.05$  versus the control group



## Malondialdehyde level in the hind paws

The hind paw samples were homogenized in lysis buffer (Thermo Fisher Scientific, MA, USA). The malondialdehyde (MDA) levels in these samples were determined using ELISA kit (USCN Business Co., Ltd., Wuhan, China) according to the manufacturer's instructions.

## Measurement of Nox activity

The Nox activity in the hind paws was measured by enhanced lucigenin chemiluminescence. Briefly, NADPH (100  $\mu$ M) was added to the media as a substrate to react with Nox and generate superoxide anions. The light emission produced during the reaction of lucigenin (5  $\mu$ M) with superoxide anions was measured using a microplate reader (BioTek, VT, USA) once every minute for 10 min. The values representing the Nox activity were expressed as the mean light units (MLU) per minute per milligram of protein.

## Measurement of superoxide anions

The superoxide anion levels in the hind paws were determined by lucigenin-derived chemiluminescence. Briefly, the dark-adapted lucigenin (5  $\mu$ M) was added to each sample

to produce photon emission, which was measured by a microplate reader (BioTek, VT, USA) once every minute for 10 min. The values representing the superoxide anion levels were expressed as the MLU per minute per milligram of protein.

## Statistical analyses

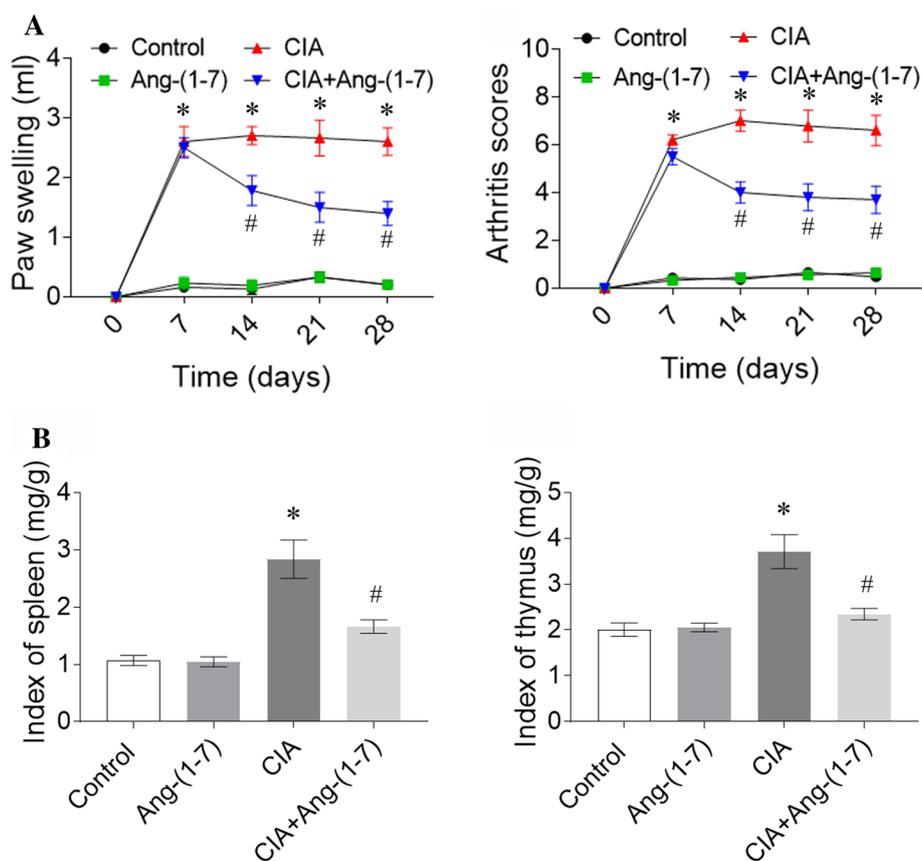
Data were presented as means  $\pm$  standard error of the mean (SE) and analyzed using GraphPad Prism 7.0 (GraphPad software Inc., CA, USA). Statistics were obtained using one-way or two-way analysis of variance (ANOVA), followed by Bonferroni test for post hoc analysis when multiple comparisons were made. A two-tailed *P* value of  $<0.05$  was considered to be statistically significant.

## Results

### The expression of ACE2/Ang-(1-7)/MasR in CIA rats

The levels of Ang-(1-7) in the serum were reduced in CIA rats (Fig. 1a). The expression of ACE2 in the hind paw of CIA rats was lower than that in the control rats (Fig. 1b, c).

**Fig. 2** Effects of angiotensin (Ang)-(1-7) on paw swelling and arthritis scores, and spleen and thymus indexes in collagen-induced arthritis (CIA) rats. **a** Ang-(1-7) treatment attenuated paw swelling and arthritis scores of CIA rats. **b** Ang-(1-7) treatment reduced both spleen and thymus indexes of CIA rats. The results are expressed as means  $\pm$  SE. *N* = 8 in the control and Ang-(1-7) groups, and *N* = 12 in the CIA and CIA + Ang-(1-7) groups. \**P* < 0.05 versus the control group; #*P* < 0.05 versus the CIA group



MasR level was reduced in the hind paw of CIA rats when compared to that in control rats (Fig. 1b, d).

from day 14. Administration of Ang-(1-7) significantly reduced the increase of arthritis scores in CIA rats (Fig. 2a).

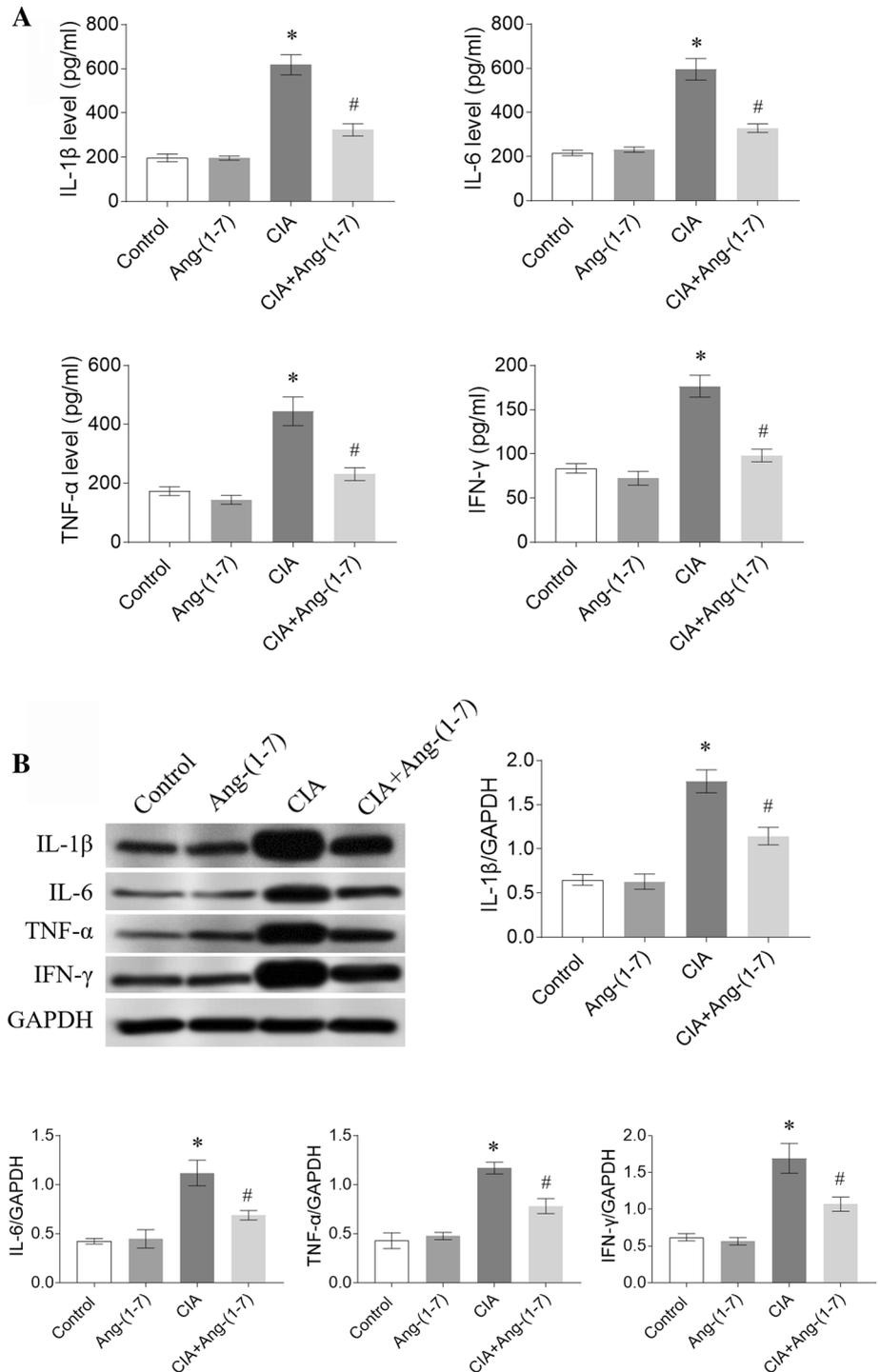
**Ang-(1-7) attenuated paw swelling and arthritis scores of CIA rats**

**Ang-(1-7) improved spleen and thymus indexes of CIA rats**

Treatment with Ang-(1-7) significantly suppressed the paw swelling of CIA rats and markedly inhibited paw edema

The indexes of spleen and thymus in CIA rats were significantly increased as compared to control rats. Ang-(1-7)

**Fig. 3** Effects of angiotensin (Ang)-(1-7) on inflammatory cytokines in collagen-induced arthritis (CIA) rats. **a** Ang-(1-7) treatment inhibited the increase of interleukin (IL)-1 $\beta$ , IL-6, tumor necrosis factor (TNF)- $\alpha$ , and interferon (IFN)- $\gamma$  in the serum of CIA rats. **b** Ang-(1-7) treatment inhibited the increase of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and IFN- $\gamma$  in the hind paw of CIA rats. The results are expressed as means  $\pm$  SE. *N* = 8 in the control and Ang-(1-7) groups, and *N* = 12 in the CIA and CIA + Ang-(1-7) groups. \**P* < 0.05 versus the control group; #*P* < 0.05 versus the CIA group



administration reduced the increase of spleen and thymus indexes of the CIA rats (Fig. 2b).

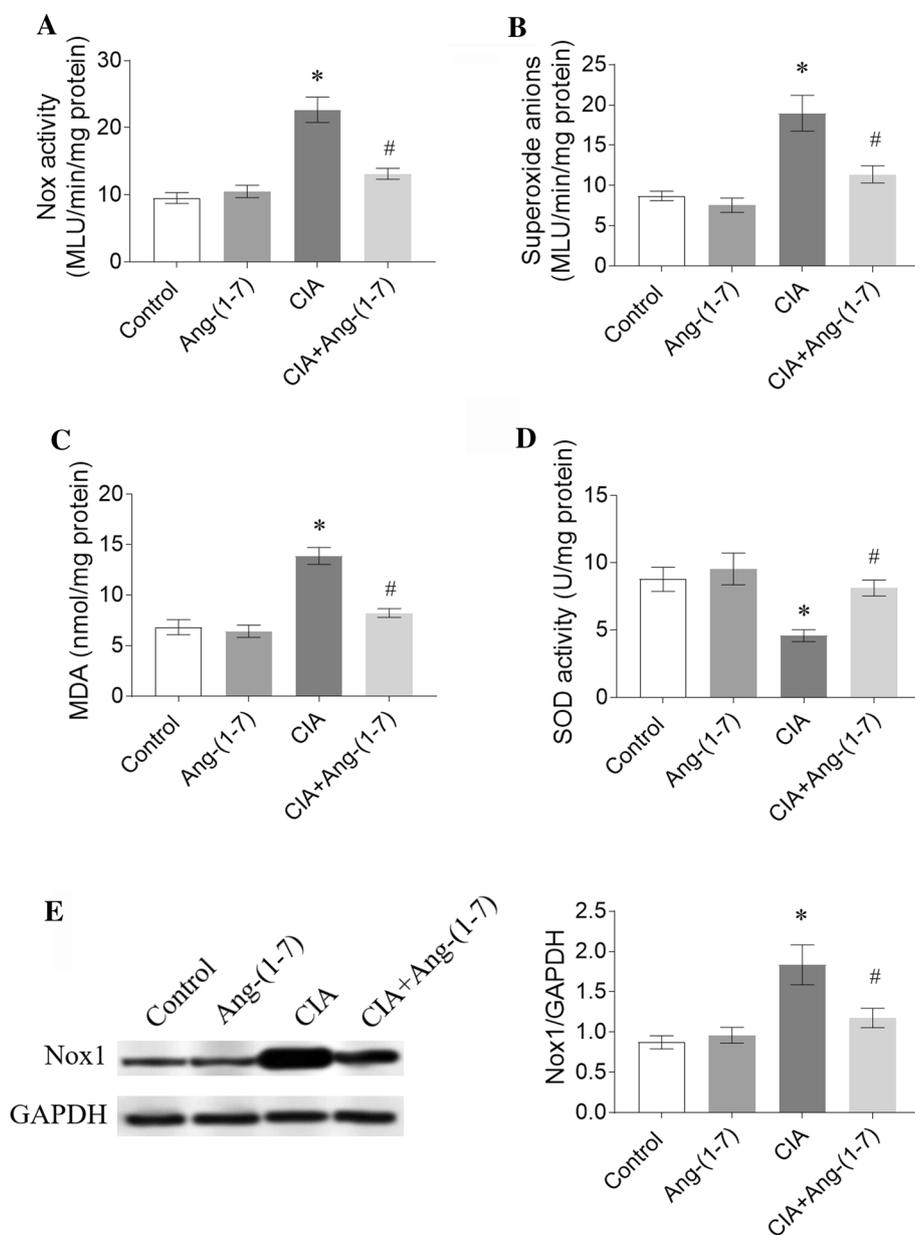
### Ang-(1-7) attenuated the increased inflammatory cytokine levels

The levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$  and IFN- $\gamma$  in the serum of CIA rats were significantly elevated when compared to the control rats. Administration of Ang-(1-7) significantly reduced these increases in the serum of CIA rats (Fig. 3a). The expressions of IL-1 $\beta$ , IL-6, TNF- $\alpha$  and IFN- $\gamma$  in the hind paw of CIA rats were increased when compared to the control rats, and these increases were inhibited after treatment with Ang-(1-7) (Fig. 3b).

### Ang-(1-7) attenuated oxidative stress in the CIA rats

The Nox activity in the hind paw was enhanced in CIA rats, and the enhancement was inhibited by treating with Ang-(1-7) (Fig. 4a). The levels of superoxide anions were increased in the hind paw of CIA rats, which was suppressed by Ang-(1-7) administration (Fig. 4b). Treatment with Ang-(1-7) attenuated the increase in MDA levels of the hind paw of CIA rats (Fig. 4c). The weakened SOD activity in the hind paw of CIA rats was improved by Ang-(1-7) treatment (Fig. 4d). Nox1 level was increased in the hind paw of CIA rats, which was reduced by Ang-(1-7) treatment (Fig. 4e).

**Fig. 4** Effects of angiotensin (Ang)-(1-7) on oxidative stress in collagen-induced arthritis (CIA) rats. **a** Ang-(1-7) treatment inhibited the increase of NADPH oxidase (Nox) activity in the hind paw of CIA rats. **b** Ang-(1-7) treatment inhibited the increase of superoxide anions in the hind paw of CIA rats. **c** Ang-(1-7) treatment inhibited the increase of malondialdehyde (MDA) in the hind paw of CIA rats. **d** Ang-(1-7) treatment inhibited the increase of superoxide dismutase (SOD) activity in the hind paw of CIA rats. **e** Ang-(1-7) treatment inhibited the increase of Nox1 expression in the hind paw of CIA rats. The results are expressed as means  $\pm$  SE.  $N=8$  in the control and Ang-(1-7) groups, and  $N=12$  in the CIA and CIA + Ang-(1-7) groups. \* $P < 0.05$  versus the control group; # $P < 0.05$  versus the CIA group



### Nox1 overexpression increased oxidative stress

Nox1 expression was elevated in the thymus of rats after treating with Ad-Nox1 (Fig. 5a). Ad-Nox1 increased the levels of Nox1 in the spleens of rats (Fig. 5b). Nox1 expression in the hind paw was significantly elevated in rats treated with Ad-Nox1 (Fig. 5c). Nox1 overexpression enhanced Nox activity, increased superoxide anions level, and elevated MDA expression in the hind paw of the rats (Fig. 5d).

### The effects of Nox1 overexpression on paw swelling and arthritis scores in CIA rats

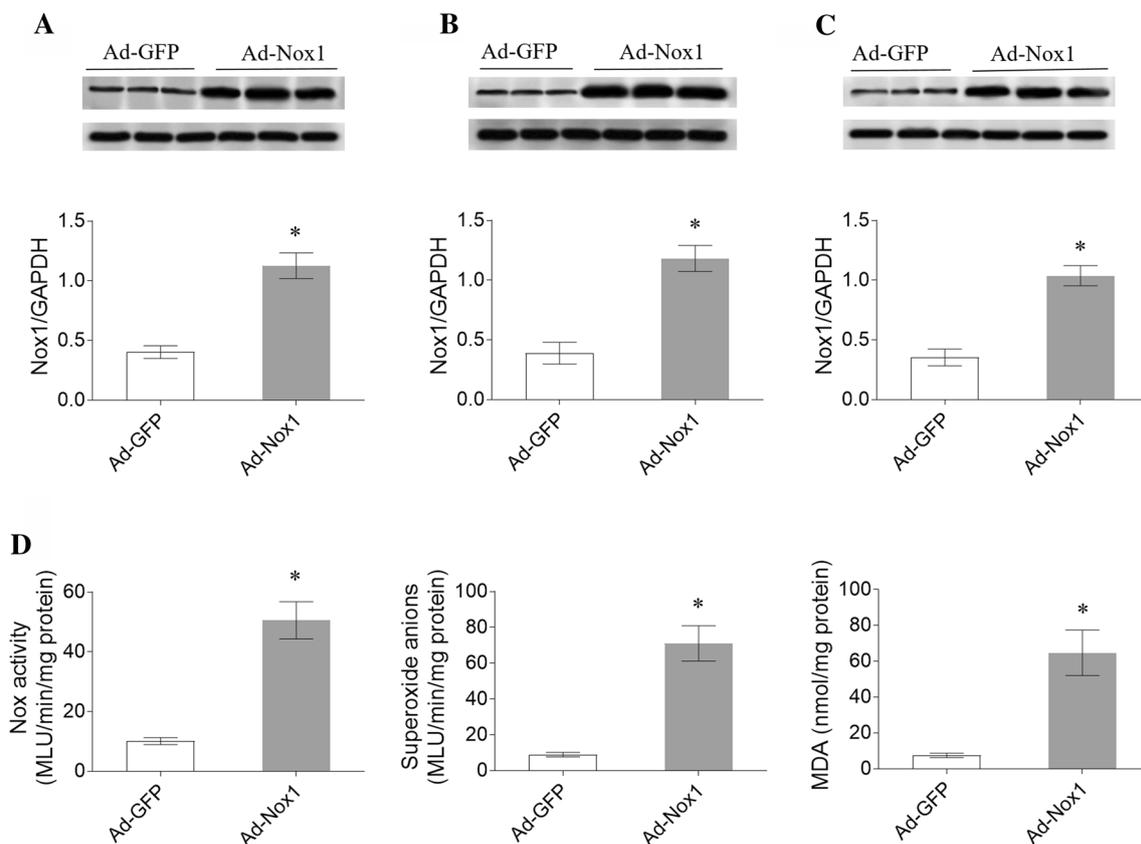
Nox1 overexpression further increased the paw swelling and arthritis scores of CIA rats. Nox1 overexpression reversed the effects of Ang-(1-7) on inhibiting paw swelling in CIA rats. Moreover, Nox1 overexpression attenuated Ang-(1-7)-induced decrease of arthritis scores in CIA rats (Fig. 6a).

### The effects of Nox1 overexpression on the spleen and thymus indexes in CIA rats

Nox1 overexpression further increased the spleen and thymus indexes of CIA rats. The increased spleen index in CIA rats was inhibited by Ang-(1-7) treatment, which was reversed by Nox1 overexpression. The decrease of thymus index in CIA rats was induced by Nox1 overexpression blocked Ang-(1-7) (Fig. 6b).

### The effects of Nox1 overexpression on inflammatory cytokine levels in CIA rats

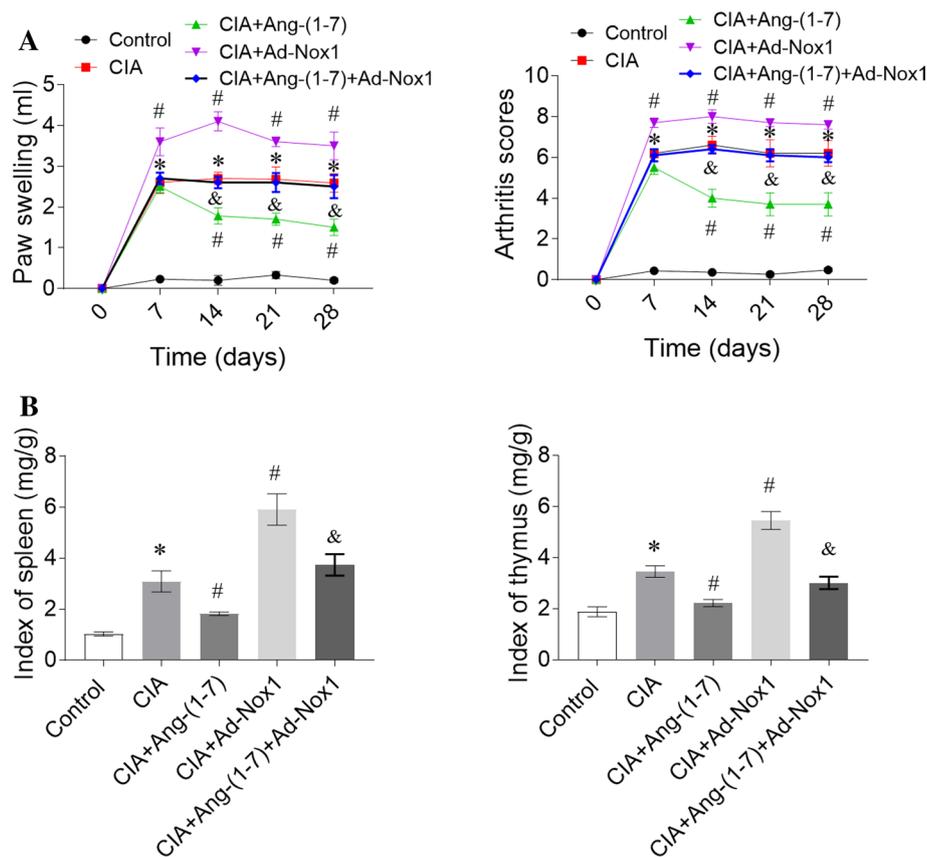
Ang-(1-7) treatment significantly reduced the increased levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$  and IFN- $\gamma$  in the serum of CIA rats. Nox1 overexpression further increased the levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$  and IFN- $\gamma$  in the serum of CIA rats. Nox1 overexpression reversed the inhibitory effects of Ang-(1-7) on the increased levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$  and



**Fig. 5** Effects of recombinant adenoviral vectors harboring NADPH oxidase 1 (Nox1) (Ad-Nox1) on oxidative stress. **a** Nox1 expression was increased in the thymus of rat treated with Ad-Nox1. **b** Nox1 expression was increased in the spleens of rats treated with Ad-Nox1. **c** Nox1 expression was increased in the hind paws of rats treated with

Ad-Nox1. **d** Treatment with Ad-Nox1 increased the levels of Nox activity, superoxide anions and malondialdehyde (MDA) in the hind paws of CIA rats. The results are expressed as means  $\pm$  SE.  $N=8$  for each group. \* $P<0.05$  versus the Ad-GFP group

**Fig. 6** Effects of NADPH oxidase 1 (Nox1) overexpression on paw swelling and arthritis scores, and spleen and thymus indexes in collagen-induced arthritis (CIA) rats. **a** Nox1 overexpression reversed the attenuating effects of angiotensin (Ang)-(1–7) on paw swelling and arthritis scores in CIA rats. **b** Nox1 overexpression reversed the attenuating effects of Ang-(1–7) on the spleen and thymus indexes of CIA rats. The results are expressed as means  $\pm$  SE.  $N=8$  in the control group, and  $N=12$  in the CIA, CIA + Ang-(1–7), CIA + Ad-Nox1 and CIA + Ang-(1–7) + Ad-Nox1 groups. \* $P < 0.05$  versus the control group; # $P < 0.05$  versus the CIA group; & $P < 0.05$  versus the CIA + Ang-(1–7) groups



IFN- $\gamma$  in the serum of CIA rats (Fig. 7a). Moreover, Nox1 overexpression further increased the levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$  and IFN- $\gamma$  in the hind paw of CIA rats. Nox1 overexpression reversed the effects of Ang-(1–7) in attenuating the increase of IL-1 $\beta$ , IL-6, TNF- $\alpha$  and IFN- $\gamma$  in the hind paw of CIA rats (Fig. 7b).

## Discussion

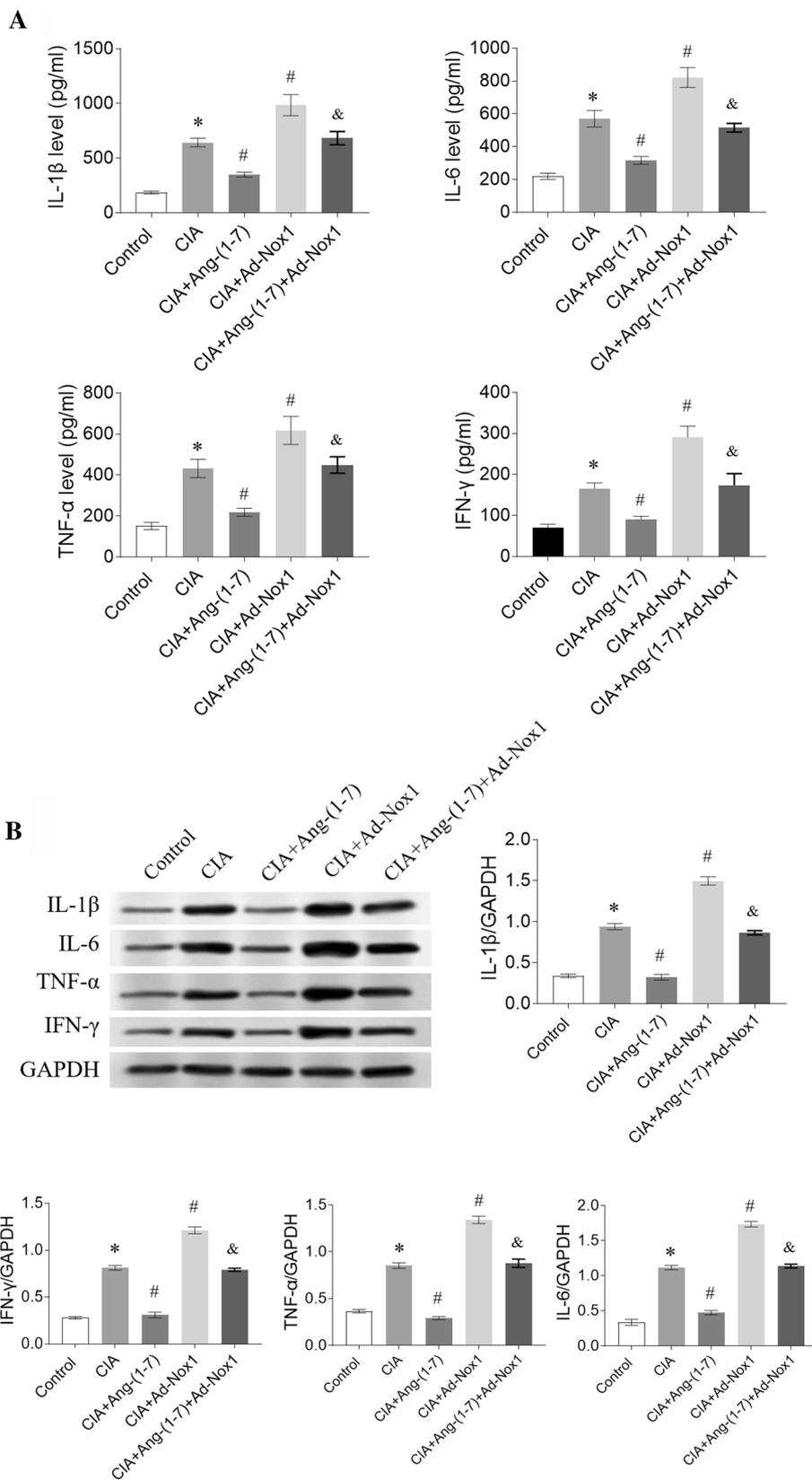
In the present study, the results showed that ACE2/Ang-(1–7)/MasR levels were reduced in CIA rats. Ang-(1–7) had anti-arthritis effects on CIA rats, as it attenuated paw edema and arthritis scores, and reduced the indexes of spleen and thymus in CIA rats when compared to those in the control rats. The current data also demonstrated that Ang-(1–7) inhibited the levels of inflammatory cytokines of CIA rats. In addition, enhanced oxidative stress in CIA rats were reversed by treatment with Ang-(1–7). Ang-(1–7) treatment reduced Nox1-induced oxidative stress in CIA. Furthermore, Nox1 overexpression inhibited the effects of Ang-(1–7) on improving arthritis of CIA rats.

Three axes of RAS family are identified, which included ACE/Ang II/AT1R, ACE2/Ang-(1–7)/MasR and alaman-dine/Mas-related G protein-coupled receptor, member D

(MrgD) (Feng et al. 2020; Li et al. 2018; Lautner et al. 2013). The expression levels of renin and ACE were elevated in the synovial tissues of patients with RA (Wu et al. 2020). ACE expression was increased and ACE2 expression was reduced in the heart of adjuvant-induced arthritis rats as compared to those in the healthy rats (Hanafy et al. 2011). Our present study showed that ACE2 expression was reduced in the hind paw of RA rats. Collagen treatment reduced the levels of Ang-(1–7) in the rats, and also the expression of MasR was reduced in mice with RA. These results indicated that ACE2/Ang-(1–7)/MasR axis was downregulated in RA rats. Exogenous administration of Ang-(1–7) or upregulation ACE2 or MasR might act as therapeutic strategies for treating RA.

Studies have shown that inhibition of renin, ACE or AT1R prevent or delay the development of arthritis (Wu et al. 2019; Sakuta et al. 2010). Activation of Mas receptors decreases cytokine production and neutrophil influx, causing significant amelioration of arthritis in rats and mice with arthritis (da Silveira et al. 2010). In the current study, Ang-(1–7) improved paw edema, and reduced arthritis scores and indexes of spleen and thymus in CIA rats. These results demonstrated the potential role of Ang-(1–7) in treating arthritis.

**Fig. 7** Effects of NADPH oxidase 1 (Nox1) overexpression on inflammatory cytokines in collagen-induced arthritis (CIA) rats. **a** Nox1 overexpression reversed the blocking effects of angiotensin (Ang)-(1-7) on the increased levels of interleukin (IL)-1 $\beta$ , IL-6, tumor necrosis factor (TNF)- $\alpha$ , and interferon (IFN)- $\gamma$  in the serum of CIA rats. **b** Nox1 overexpression reversed the blocking effects of Ang-(1-7) on increased levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and IFN- $\gamma$  in the hind paw of CIA rats. The results are expressed as means  $\pm$  SE.  $N=8$  in the control group, and  $N=12$  in the CIA, CIA + Ang-(1-7), CIA + Ad-Nox1 and CIA + Ang-(1-7) + Ad-Nox1 groups. \* $P < 0.05$  versus the control group; # $P < 0.05$  versus the CIA group; & $P < 0.05$  versus the CIA + Ang-(1-7) groups



Ang-(1–7) might drive anti-inflammatory effects through its receptor in lipopolysaccharide-induced macrophages (Jiang et al. 2019). Ang-(1–7) via its Mas receptor acts as an anti-inflammatory pathway in allergic asthma (El-Hashim et al. 2012). Ang-(1–7) reversed the effects of arthritis on TNF- $\alpha$  expression, showing no effects on IL-1 $\beta$  level in the aorta of RA rats (Acikalin et al. 2016). In our present study, Ang-(1–7) administration significantly reduced the levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and IFN- $\gamma$  in the CIA rats, suggesting that the suppressive effects of Ang-(1–7) on RA in CIA rats could be attributed to the down-regulation of inflammatory mediators.

The antioxidants and oxidative stress play important roles during the disease process of RA (Quinonez-Flores et al. 2016). ROS production is shown to be significantly decreased in adipocytes treated with Ang-(1–7) (Liu et al. 2012). Ang-(1–7) administration also inhibited diabetes-induced ROS production by increasing SOD expression and decreasing p22-phox levels, leading to a significant reduction in 3-nitrotyrosine formation in diabetic bone marrow (Mordwinkin et al. 2012). In the current study, our results showed that Nox activity, superoxide anions and MDA were enhanced, and SOD activity was attenuated in CIA rats, and these changes were reversed by Ang-(1–7) treatment. These results indicated that CIA induced an imbalance between antioxidants and oxidants in rats, and Ang-(1–7) treatment improved RA by inhibiting oxidative stress. Besides, Ang-(1–7) was found to inhibit the increase of Nox1 in CIA rats. Nox1 overexpression inhibited the attenuating effects of Ang-(1–7) on paw swelling, arthritis scores, indexes of spleen and thymus, inflammatory cytokine levels in CIA rats. The results demonstrated that Nox1 increased oxidative stress in arthritis, and Ang-(1–7) treatment reduced Nox1-induced oxidative stress. Ang-(1–7) attenuated the oxidative stress via reducing Nox1 expression to improve RA.

In conclusion, Ang-(1–7) improved arthritis and attenuated inflammatory cytokines in CIA rats. In addition, CIA induced an imbalance between oxidants and antioxidants, and Ang-(1–7) treatment attenuated Nox1-induced oxidative stress in CIA. Ang-(1–7) improved arthritis via reducing Nox1 expression to attenuate oxidative stress.

## Compliance with ethical standards

**Conflict of interest** All authors declare that no competing financial interests exist.

**Ethical approval** All procedures were approved by the Experimental Animal Care and Use Committee of Nanjing Medical University and conducted in accordance with the Guide for the Care and Use of Laboratory Animals. The ethical approval number was 17062846 and the study was approved in June 2017.

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