Redox Biology 12 (2017) 377–388

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Research paper

CO-releasing molecules CORM2 attenuates angiotensin II-induced human aortic smooth muscle cell migration through inhibition of ROS/IL-6 generation and matrix metalloproteinases-9 expression

Ming-Horng Tsai[a,b], Chiang-Wen Lee[c,d,e,*], Lee-Fen Hsu[f], Shu-Yu Li[g], Yao-Chang Chiang[h], Ming-Hsueh Lee[i], Chun-Han Chen[j], Hwey-Fang Liang[c], Jia-Mei How[k], Pey-Jium Chang[b], Ching-Mei Wu[l], I-Ta Lee[k,m,**]

[a] Department of Pediatrics, Division of Neonatology and Pediatric Hematology/Oncology, Chang Gung Memorial Hospital, Yunlin, Taiwan
[b] Graduate Institute of Clinical Medical Science, College of Medicine, Chang Gung University, Taiwan
[c] Department of Nursing, Division of Basic Medical Sciences, Chang Gung University of Science and Technology, Chia-Yi, Taiwan
[d] Chronic Diseases and Health Promotion Research Center, Chang Gung University of Science and Technology, Chia-Yi, Taiwan
[e] Research Center for Industry of Human Ecology, Chang Gung University of Science and Technology, Taoyuan, Taiwan
[f] Department of Respiratory Care, Chang Gung University of Science and Technology, Chiayi Campus, Chiayi, Taiwan
[g] Department of Pharmacy, College of Pharmacy & Health Care, Tajen University, Taiwan
[h] Center for Drug Abuse and Addiction, China Medical University Hospital, China Medical University, Taichung, Taiwan
[i] Division of Neurosurgery, Department of Surgery, Chang Gung Memorial Hospital, Chia-Yi 61363, Taiwan
[j] Division of General Surgery, Department of Surgery, Chang Gung Memorial Hospital at Chiayi, Chiayi, Taiwan
[k] School of Medicine, College of Medicine, China Medical University, Taichung, Taiwan
[l] Department of Sports Medicine, College of Health Care, China Medical University, Taichung, Taiwan
[m] Graduate Institute of Biomedical Sciences, College of Medicine, China Medical University, Taichung, Taiwan

ARTICLE INFO

Keywords:
Angiotensin II
Carbon monoxide
Human aortic smooth muscle cell
Inflammation
Matrix metallopeptidase-9

ABSTRACT

Ang II has been involved in the pathogenesis of cardiovascular diseases, and matrix metalloproteinase-9 (MMP-9) induced migration of human aortic smooth muscle cells (HASMCs) is the most common and basic pathological feature. Carbon monoxide (CO), a byproduct of heme breakdown by heme oxygenase, exerts anti-inflammatory effects in various tissues and organ systems. In the present study, we aimed to investigate the effects and underlying mechanisms of carbon monoxide releasing molecule-2 (CORM-2) on Ang II-induced MMP-9 expression and cell migration of HASMCs. Ang II significantly up-regulated MMP-9 expression and cell migration of HASMCs, which was inhibited by transfection with siRNA of $p47^{phox}$, Nox2, Nox4, p65, angiotensin II type 1 receptor (AT1R) and pretreatment with the inhibitors of NADPH oxidase, ROS, and NF-κB. In addition, Ang II also induced NADPH oxidase/ROS generation and $p47^{phox}$ translocation from the cytosol to the membrane. Moreover, Ang II-induced oxidative stress and MMP-9-dependent cell migration were inhibited by pretreatment with CORM-2. Finally, we observed that Ang II induced IL-6 release in HASMCs via AT1R, but not AT2R, which could further caused MMP-9 secretion and cell migration. Pretreatment with CORM-2 reduced Ang II-induced IL-6 release. In conclusion, CORM-2 inhibits Ang II-induced HASMCs migration through inactivation of suppression of NADPH oxidase/ROS generation, NF-κB inactivation and IL-6/MMP-9 expression. Thus, application of CO, especially CORM-2, is a potential countermeasure to reverse the pathological changes of various cardiovascular diseases. Further effects aimed at identifying novel antioxidant and anti-inflammatory substances protective for heart and blood vessels that targeting CO and establishment of well-designed in vivo models properly evaluating the efficacy of these agents are needed.

* Correspondence to: Department of Nursing, Division of Basic Medical Sciences, and Chronic Diseases and Health Promotion Research Center, Chang Gung University Of Science and Technology, Chia-Yi, Taiwan.
** Correspondence to: School of Medicine, College of Medicine, China Medical University, No. 91, Hsueh-Shih Road, Taichung 40402, Taiwan, R.O.C.
E-mail addresses: cwlee@mail.cgust.edu.tw (C.-W. Lee), ita0128@mail.cmu.edu.tw (I.-T. Lee).

http://dx.doi.org/10.1016/j.redox.2017.02.019
Received 28 January 2017; Received in revised form 22 February 2017; Accepted 23 February 2017
Available online 01 March 2017

1. Introduction

Angiotensin II (Ang II) plays an important role in the pathogenesis of several cardiovascular diseases including cardiomyopathy, coronary artery disease, atherosclerosis and vascular injury [1]. Ang II is the key effector peptide of the rennin-angiotensin system (RAS) and exerts the vasculature remodeling through mechanisms of promoting cell proliferation, fibrosis, oxidative stress and inflammation [2,3]. All these processes lead to initiation of vascular damage. There have been several studies that demonstrated the beneficial effect of inhibition of the RAS system, or directly inhibition of Ang II, on the prevention or treatment of cardiovascular disease [4–6].

After vascular damage, the pathogenesis of these cardiovascular diseases includes the degradation of the extracellular matrix, through various inflammatory reactions, generation of reactive oxygen species (ROS) and matrix metalloproteinase (MMPs) [7,8]. The relationship between Ang II and ROS has been the focus of many studies since decades ago and Ang II has been demonstrated to exert its vascular remodeling effects through NADPH oxidase-derived generation of ROS [9–11]. Ang II activates signaling response through the Ang II type 1 receptor (AT1R) and NADPH oxidase, which lead to ROS generation in vascular smooth muscle cell (VSMC), and subsequently, the proliferative, migratory and inflammatory responses of VSMC [9,12].

Carbon monoxide (CO) is endogenously produced in cells and tissues as the byproduct of heme oxygenase (HO) enzymes after heme catalytic activity [13,14]. Although CO is toxic to human bodies at high concentration, low-dose exogenous CO (approximately 250–500 ppm) treatment has been demonstrated to have protective functions, anti-inflammatory and antiapoptotic effects in various human diseases [15–18]. Furthermore, accumulating studies over the past decade have shown that low concentration of CO, or CO-releasing molecules (CORMs), can confer several molecular mechanisms, including eliminating microbes, regulating cell death and organ dysfunction, and promote immune response through resolving mediator-heme oxygenase-1 circuits [19], activation of p38 MAPK, enhanced PPAR-γ singaling, and suppression of TLRs translocation [13,16,20]. Among them, the most characterized CO-RMs is the lipid-soluble tricarbonyldichlororuthenium (II) dimmer (CORM-2) [21–23]. We hypothesized that CORM-2 might be potentially as an anti-inflammatory modulator and therapeutic agent of vascular inflammation.

CORM-2 has been proven to inhibit MMP-1 and MMP-2 expression in human lung epithelial cell [24]; however, the most important MMPs involved in cardiovascular diseases is MMP-9 [25]. MMP-9 plays an important role of vascular remodeling and inflammation through directly cleaving the extracellular matrix and activation of various chemokines and cytokines [25]. MMP-9 inhibition has been proven significantly beneficial in multiple animal models of cardiovascular disease [26–28]. In this present study, we aimed to investigate the therapeutic potential of CORM2 on inhibition of VSMC migration, a hallmark of cardiovascular disease, and the underlying mechanisms of CORM2 on MMP-9 inhibition.

2. Results

2.1. Ang II induces MMP-9-dependent cell migration

Here, we examined the effect of Ang II on the cell viability of human aortic smooth muscle cells (HASMCs). As shown in Fig. 1A, Ang II (1, 5, and 10 μM) had no effects on the cell viability of HASMCs. However, treatment of 100 μM Ang II for 24 h significantly increased the cell viability of HASMCs. Ang II acts via binding to receptor types AT1R and AT2R [12]. In our study, we found that 10 μM Ang II markedly induced AT1R and AT2R mRNA levels in HASMCs (Fig. 1B). We further determined the effect of Ang II treatment on MMP-9 expression in HASMCs. As shown in Fig. 1C, cells were treated with Ang II for 2, 4, 6, 16, or 24 h, and then the protein expression of MMP-9 was determined by Western blot analysis. We showed that Ang II induced MMP-9 protein expression in a time-dependent manner. Ang II-induced MMP-9 expression was significantly increased within 6 h and continued to increase over 24 h. In addition, Ang II also induced MMP-9 mRNA levels and promoter activity in these cells (Fig. 1D and E). The biological importance of MMPs has been described in multiple cellular processes including proliferation, angiogenesis, migration, host defense, cancer invasion, and metastasis [29]. Indeed, we showed that Ang II induced migration of HASMCs, which was reduced by transfection with MMP-9 siRNA. Taken together, these data suggest that Ang II induces MMP-9-dependent migration of HASMCs.

2.2. CORM-2 inhibits Ang II-induced MMP-9 expression and cell migration

Rodriguez et al. found that induction of HO-1 or CO, but not biliverdin or bilirubin, inhibited VSMC migration [30]. Here, we examined the effect of CORM-2 on the cell viability of HASMCs. As shown in Fig. 2A, CORM-2 (1, 10, 50, and 100 μM) had no effects on the cell viability of HASMCs. To further examine the mechanisms which end metabolites of heme catabolism by HO-1, including CO, contribute to the attenuation of Ang II-mediated responses in HASMCs, cells were incubated with the CO donor, CORM-2 before the addition of Ang II. The results showed that pretreatment with CORM-2 caused a significant decrease in Ang II-induced MMP-9 concentration, pro-MMP-9 expression, cell migration, MMP-9 mRNA levels, and MMP-9 promoter activity (Fig. 2B–E). However, as shown in Fig. 2B, D and E, the addition of CO scavenger, hemoglobin, significantly reversed the attenuating effects of CORM-2 on MMP-9 concentration, cell migration, MMP-9 mRNA levels, and MMP-9 promoter activity induced by Ang II, indicated that CORM-2 contributes to protection of cells from Ang II insult through at least partly a CO-dependent attenuation of MMP-9 expression and migration of HASMCs.

2.3. Ang II induces MMP-9 expression via NADPH oxidase/ROS in HASMCs

Sun et al. found that salusin-β promotes vascular smooth muscle cell migration and intimal hyperplasia after vascular injury via ROS/NF-κB/MMP-9 pathway [31]. In addition, ROS is a family of non-phagocytic NADPH oxidases, including the prototypic Nox2 homolog-based NADPH oxidase, as well as other NADPH oxidases, such as Nox1 and Nox4. Thus we investigated whether NADPH oxidase was involved in Ang II-induced MMP-9 expression in HASMCs. As shown in Fig. 3A, transfection with Nox2 or Nox4 siRNA markedly inhibited Ang II-induced pro-MMP-9 expression. Activated NADPH oxidase is a multimeric protein complex consisting of at least three cytosolic subunits of $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$. Phosphorylation of $p47^{phox}$ leads to a conformational change allowing its interaction with $p22^{phox}$ [32]. Moreover, we showed that transfection with siRNA of $p47^{phox}$, Nox2, or Nox4 markedly inhibited Ang II-induced MMP-9 concentration in HASMCs (Fig. 3B). Ang II-enhanced migration of HASMCs was also reduced by transfection with these three siRNAs (Fig. 3C). Finally, we demonstrated that pretreatment with the inhibitor of ROS (NAC) or NADPH oxidase (DPI or APO) inhibited Ang II-induced MMP-9 mRNA levels and promoter activity in these cells (Fig. 3D). Taken together, these results suggest that Ang II induces MMP-9 expression via NADPH oxidase and ROS in HASMCs.

2.4. CORM-2 inhibits Ang II-induced NADPH oxidase activation and ROS generation

Cepinskas et al. showed that CORM-2-released CO attenuated LPS-induced production of ROS and NO [33]. Here we also investigated whether CORM-2 could inhibit Ang II-induced ROS generation and

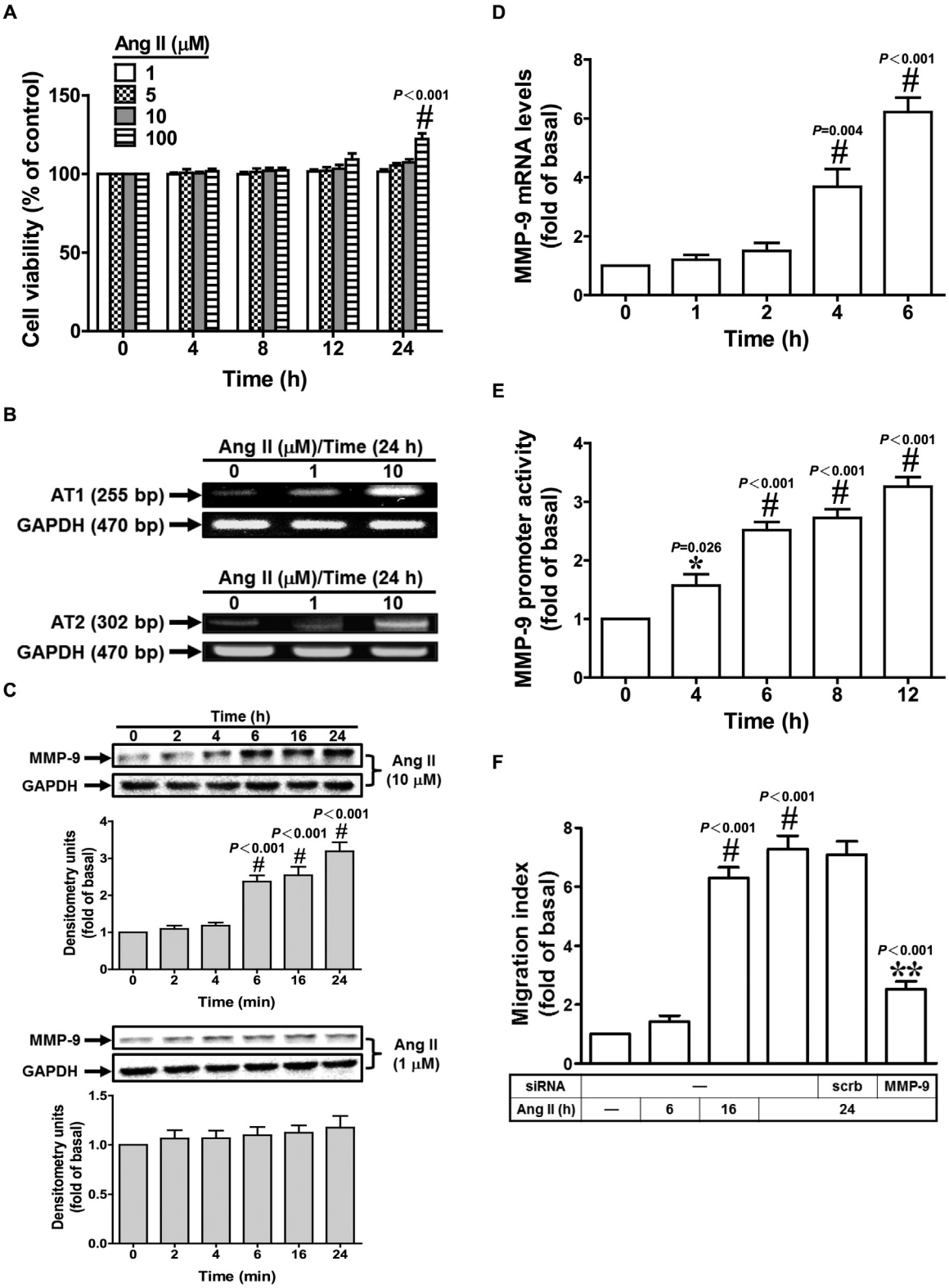

Fig. 1. *Ang II induces MMP-9-dependent cell migration.* (A) Cells were incubated with Ang II for the indicated times, and then the cell viability was determined. (B) HASMCs were treated with Ang II for 24 h, and then the mRNA levels of AT1R and AT2R were determined by RT-PCR. (C) Cells were incubated with Ang II for the indicated times, and then the protein expression of MMP-9 was determined by Western blot. (D, E) Cells were incubated with Ang II (10 μM) for the indicated times, and then the mRNA levels and promoter activity of MMP-9 were determined by real-time PCR and promoter assay, respectively. (F) Cells were incubated with Ang II (10 μM) for the indicated times or transfected with siRNA of scrambled or MMP-9, and then treated with Ang II (10 μM) for 24 h. The cell migration was determined by migration assay. Data are expressed as mean ± S.E.M. of three independent experiments. $^{*}P < 0.05$; $\#P < 0.01$, as compared with the basal level. $^{**}P < 0.01$, as compared with the cells exposed to Ang II + scrambled siRNA.

NADPH oxidase activation in HASMCs. As shown in Fig. 4A, treatment of Ang II markedly enhanced intracellular ROS production in a time-dependent manner. Moreover, transfection with siRNA of Nox2, Nox4, or $p47^{phox}$ inhibited Ang II-induced ROS generation in HASMCs (Fig. 4B). On the other hand, Ang II time-dependently induced NADPH oxidase activity in these cells (Fig. 4C). We further found that

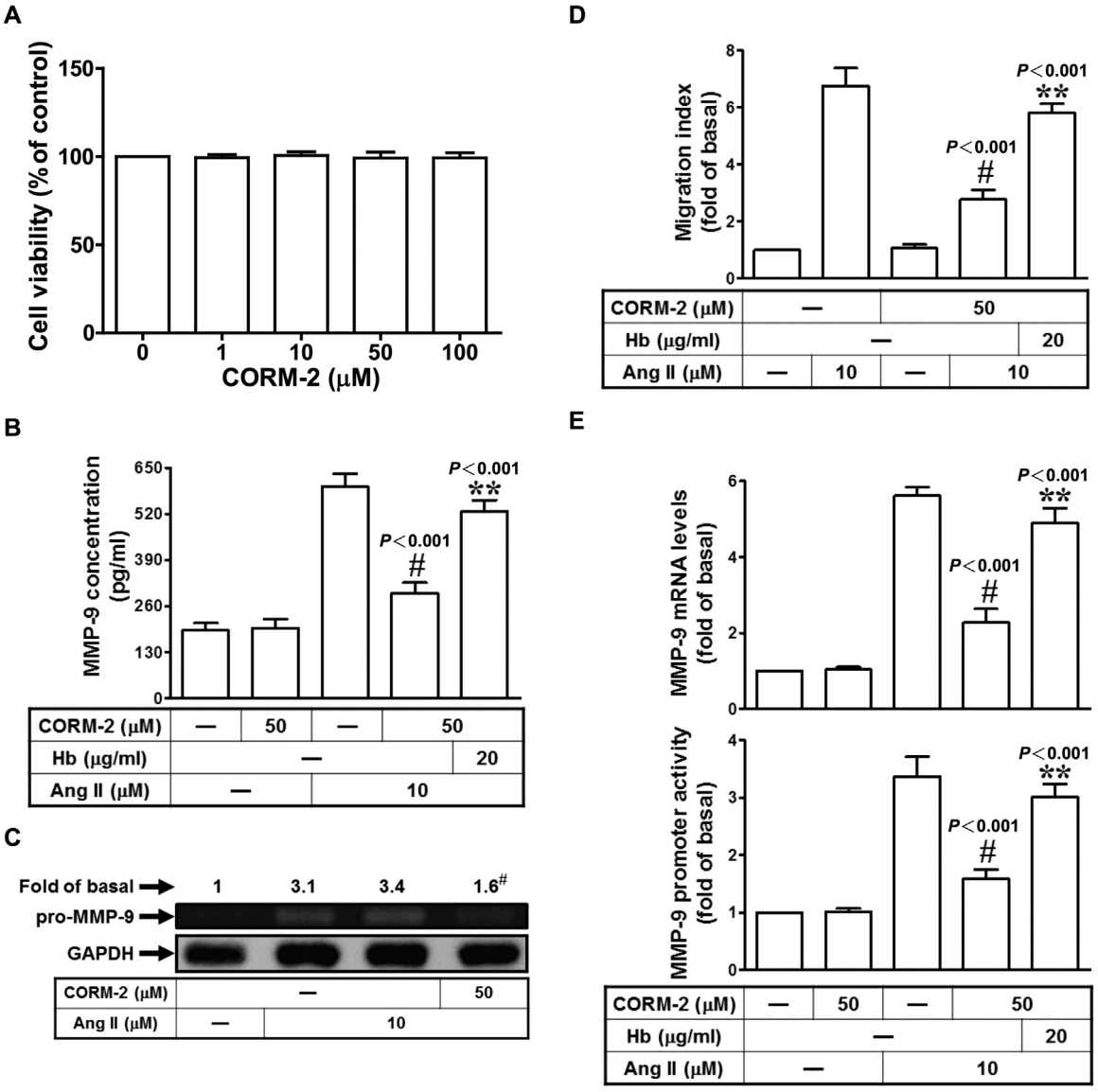

Fig. 2. *CORM-2 inhibits Ang II-induced MMP-9 expression and cell migration.* (A) Cells were incubated with CORM-2 for the indicated times, and then the cell viability was determined. (B) Cells were pretreated with CORM-2 for 2 h in the presence or absence of hemoglobin (Hb), and then treated with Ang II for 24 h. The concentration of MMP-9 was determined. (C) Cells were pretreated with CORM-2 for 2 h, and then treated with Ang II for 24 h. The conditioned media were subjected to determine MMP-9 expression by gelatin zymography. (D) Cells were pretreated with CORM-2 for 2 h in the presence or absence of Hb, and then treated with Ang II for 24 h. The cell migration was determined by migration assay. (E) Cells were pretreated with CORM-2 for 2 h in the presence or absence of Hb, and then treated with Ang II for 6 h. The mRNA levels and promoter activity of MMP-9 were determined by real-time PCR and promoter assay, respectively. Data are expressed as mean ± S.E.M. of three independent experiments. #$P < 0.01$, as compared with the cells exposed to Ang II alone. **$P < 0.01$, as compared with the cells exposed to Ang II + CORM-2.

Ang II induced $p47^{phox}$ translocation from the cytosol to the membrane, which was inhibited by pretreatment with CORM-2 (Fig. 4D). Finally, we demonstrated that CORM-2 could reduce Ang II-induced ROS generation and NADPH oxidase activity in HASMCs (Fig. 4E). Thus these data suggest that CORM-2 can reduce MMP-9 expression via inhibition of the ROS generation and NADPH oxidase activation in HASMCs.

2.5. CORM-2 reduces Ang II-induced NF-κB activation in HASMCs

NF-κB was shown to be involved in MMP-9 regulation in response to various stimulators [34,35]. ROS have been shown to regulate NF-κB activation [36]. Moreover, we showed that transfection with p65 siRNA markedly reduced Ang II-induced MMP-9 concentration and migration of HASMCs (Fig. 5A and B). In addition, we also found that pretreatment with the inhibitor of NF-κB (HLN or Bay11-7082) inhibited Ang II-induced MMP-9 mRNA levels and promoter activity in these cells (Fig. 5C). On the other hand, Ang II time-dependently induced NF-κB promoter activity (Fig. 5D), which was reduced by pretreatment with CORM-2, NAC, APO, DPI, and HLN (Fig. 5E). Finally, we showed that Ang II markedly induced NF-κB p65 activation, which was reduced by CORM-2, NAC, or HLN (Fig. 5F). Therefore we suggest that CORM-2 also can inhibit MMP-9-dependent migration via inhibition of the NF-κB activation in HASMCs.

2.6. CORM-2 reduces Ang II-induced MMP-9 expression via inhibition of the AT1R/IL-6 pathway in HASMCs

IL-6 has been shown to regulate cell migration in various cell types [37,38]. Ang II has also been shown to induce interleukin-6 (IL-6) expression in astrocytes [39]. Here we investigated whether Ang II could induce IL-6-dependent MMP-9 expression in HASMCs. As shown in Fig. 6A, Ang II induced IL-6 release in a time-dependent manner. Moreover, we found that Ang II-induced MMP-9 concentra-

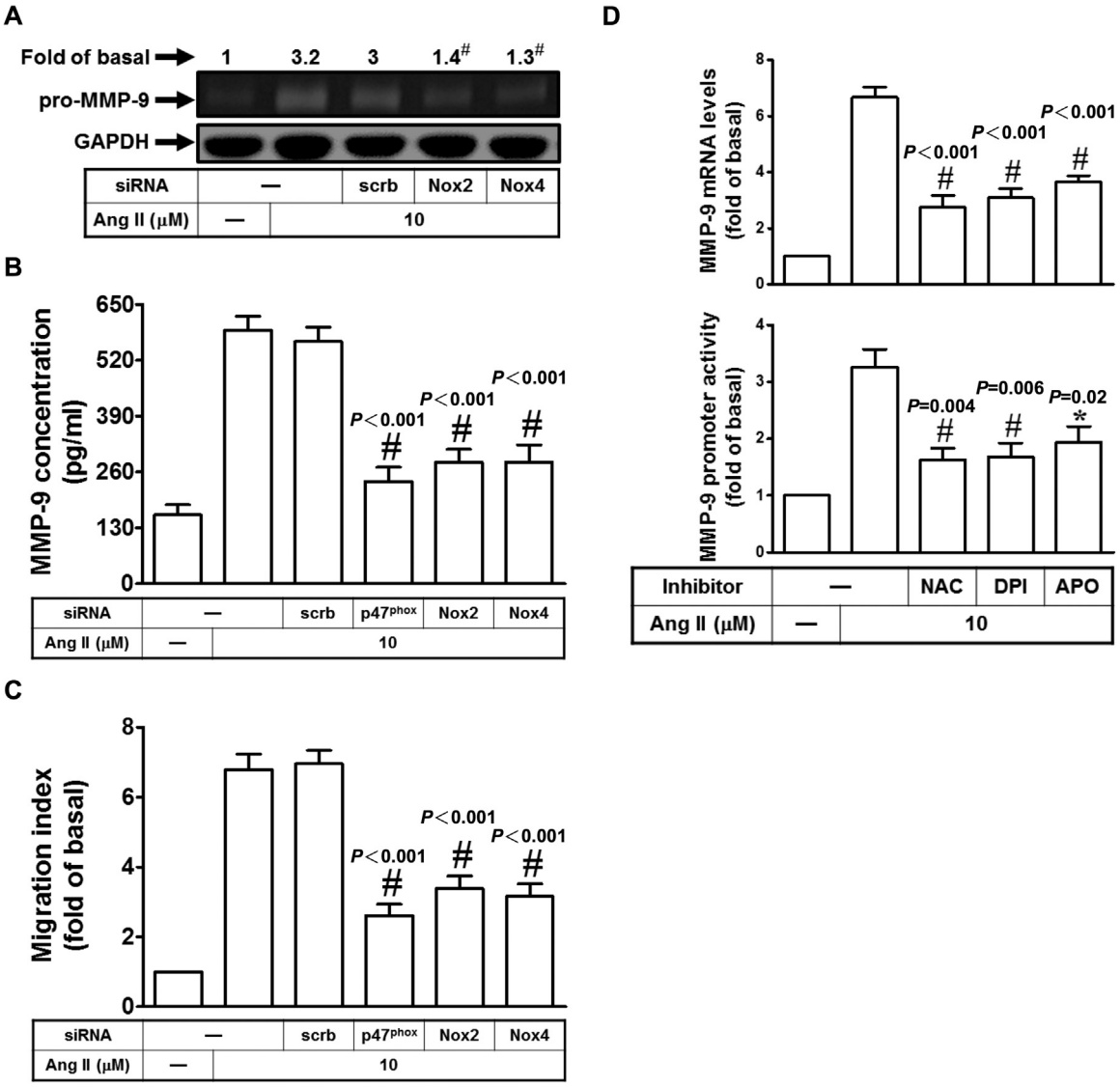

Fig. 3. *Ang II induces MMP-9 expression via NADPH oxidase/ROS in HASMCs.* (A) Cells were transfected with siRNA of scrambled, Nox2, or Nox4, and then treated with Ang II for 24 h. The conditioned media were subjected to determine MMP-9 expression by gelatin zymography. (B) Cells were transfected with siRNA of scrambled, $p47^{phox}$, Nox2, or Nox4, and then treated with Ang II for 24 h. The concentration of MMP-9 was determined. (C) Cells were transfected with siRNA of scrambled, $p47^{phox}$, Nox2, or Nox4, and then treated with Ang II for 24 h. The cell migration was determined by migration assay. (D) Cells were pretreated with NAC, DPI, or APO for 1 h, and then treated with Ang II for 6 h. The mRNA levels and promoter activity of MMP-9 were determined by real-time PCR and promoter assay, respectively. Data are expressed as mean ± S.E.M. of three independent experiments. $*P < 0.05$; $\#P < 0.01$, as compared with the cells exposed to Ang II + scrambled siRNA (A-C) or Ang II alone (D).

tion and migration of HASMCs were reduced by transfection with IL-6 siRNA (Fig. 6B and C). On the other hand, we showed that pretreatment with HLN, NAC, APO, or CORM-2 could inhibit Ang II-induced IL-6 release (Fig. 6D). Ang II acts via binding to receptor types *AT1R* and *AT2R* [12]. Finally, we demonstrated that Ang II-induced Il-6 and MMP-9 production was reduced by transfection with AT1R siRNA, but not AT2R siRNA in HASMCs (Fig. 6E and F). Taken together, we suggest that CORM-2 inhibits Ang II-induced MMP-9 expression via inhibition of the AT1R-dependent IL-6 release in HASMCs.

3. Discussion

In this study, we demonstrated that Ang II induced migration of HASMCs is through AT1R and activation of NADPH oxidase/ROS/NF-κB/IL-6-dependent MMP-9 expression. We also demonstrated CORM-2 inhibited Ang II-induced NADPH oxidase/ROS generation, NF-κB activation, IL-6 release, and MMP-9-dependent HASMCs migration. We found the benefits of CORM-2 in reducing the HASMCs, a hallmark of vascular remodeling, so administration of exogenous CO may serve as a potential therapeutic strategy for many cardiovascular diseases. Therefore, further efforts aimed at identifying novel antioxidant and anti-inflammatory substances protective for heart and blood vessels that targeting CO and establishment of well-designed *in vivo* models properly evaluating the efficacy of these agents are needed.

Ang II is previously identified as a potent vasoconstrictor but now it has been recognized as the key vasoactive peptide that plays an important role in the pathogenesis of vascular disease, including cell growth, inflammation, migration, and apoptosis of VSMCs [40,41]. It is well known that Ang II contributes to hypertension and atherosclerosis. Recently, there have been more studies investigating the molecular mechanisms and effects of Ang II on the blood vessels, including induction of Egr-1 expression [42], AT1R and p38MAPK pathway interactions [43], sphingosine-1-phosphate induced signaling axis [44], and Toll-like receptor 4 (TLR4) signaling pathway [45]. Although it has been demonstrated that Ang II can induce MMP-9 expression in VSMCs via a NF-κB dependent pathway [46], we found the NF-κB binding activity is through AT1R, but not AT2R. Furthermore, the effect of Ang II to induce IL-6 and ROS generation on the HASMCs was

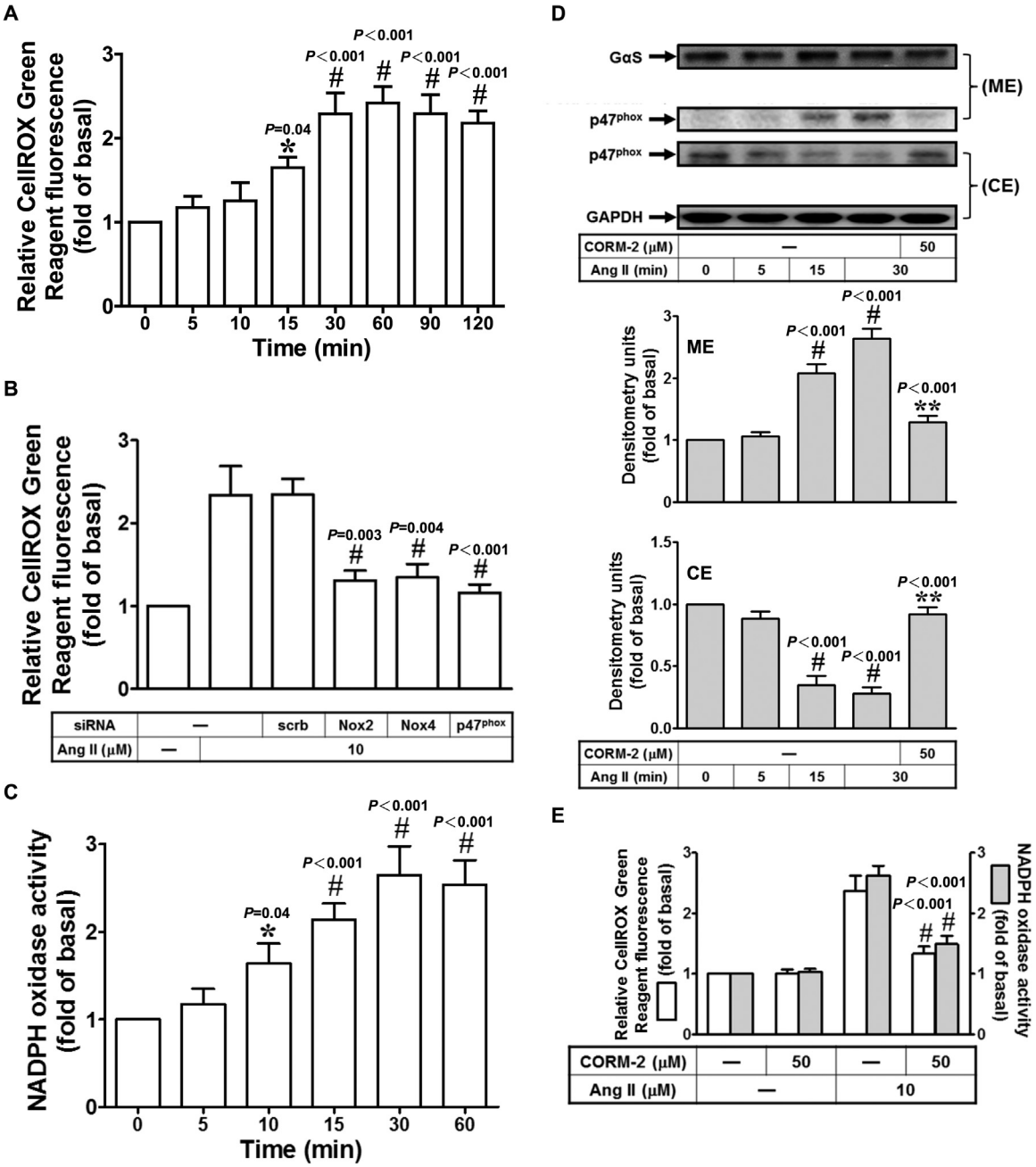

Fig. 4. *CORM-2 inhibits Ang II-induced NADPH oxidase activation and ROS generation.* (A) Cells were treated with 10 μM Ang II for the indicated times, and then the generation of ROS was determined. (B) Cells were transfected with siRNA of scrambled, $p47^{phox}$, Nox2, or Nox4, and then treated with Ang II for 60 min. The generation of ROS was determined. (C) Cells were treated with 10 μM Ang II for the indicated times, and then the activity of NADPH oxidase was determined. (D) Cells were treated with 10 μM Ang II for the indicated times or pretreated with CORM-2 for 2 h, and then treated with Ang II for 30 min. The cytosolic and membrane fractions were prepared and subjected to Western blot using an anti-$p47^{phox}$ antibody. GAPDH and GαS were used as a marker protein for cytosolic and membrane fractions, respectively. (E) Cells were pretreated with CORM-2 for 2 h, and then treated with Ang II for 60 min (for ROS generation) or 30 min (for NADPH oxidase activity). The ROS generation and NADPH oxidase activity were determined. Data are expressed as mean ± S.E.M. of three independent experiments. *$P < 0.05$; #$P < 0.01$, as compared with the basal level (A, C, D). #$P < 0.01$, as compared with the cells exposed to Ang II + scrambled siRNA (B). **$P < 0.01$, as compared with the cells exposed to Ang II alone (D). #$P < 0.01$, as compared with the cells exposed to Ang II alone (E).

also found in the central nervous system [47]. Therefore, more detailed elucidation of the mechanisms involved in Ang II pro-inflammatory effects in the cardiovascular system can contribute to the development of novel therapeutic strategies, which can also treat hypertension through central nervous system [48].

Unbalanced production of ROS has been reported in various pathological conditions of cardiovascular systems [49–51]. NADPH oxidase in the plasma membrane and mitochodria are the major sources of ROS. In this report we found Ang II triggers the formation of ROS via AT1R and NADPH oxidase in the plasma membrane, with a subsequent increase in oxidative damage. In this study, we also found that Ang II induced MMP-9 expression and cell migration is related to NADPH oxidase activation and intracellular ROS production. In HASMCs challenged with CORM-2, Ang II-enhanced ROS generation and NADPH oxidase activity in HASMCs were attenuated. Thus CORM-2 may inhibit MMP-9 expression through this antioxidant property.

Heme oxygenase-1 (HO-1) and its enzymatic product, carbon monoxide (CO), has been demonstrated using transgenic animal models to show its protective effects against the inflammatory response and oxidative stress in rats since more than decades ago [52–54]. CORMs have been proven as a potential therapeutic regimen in several

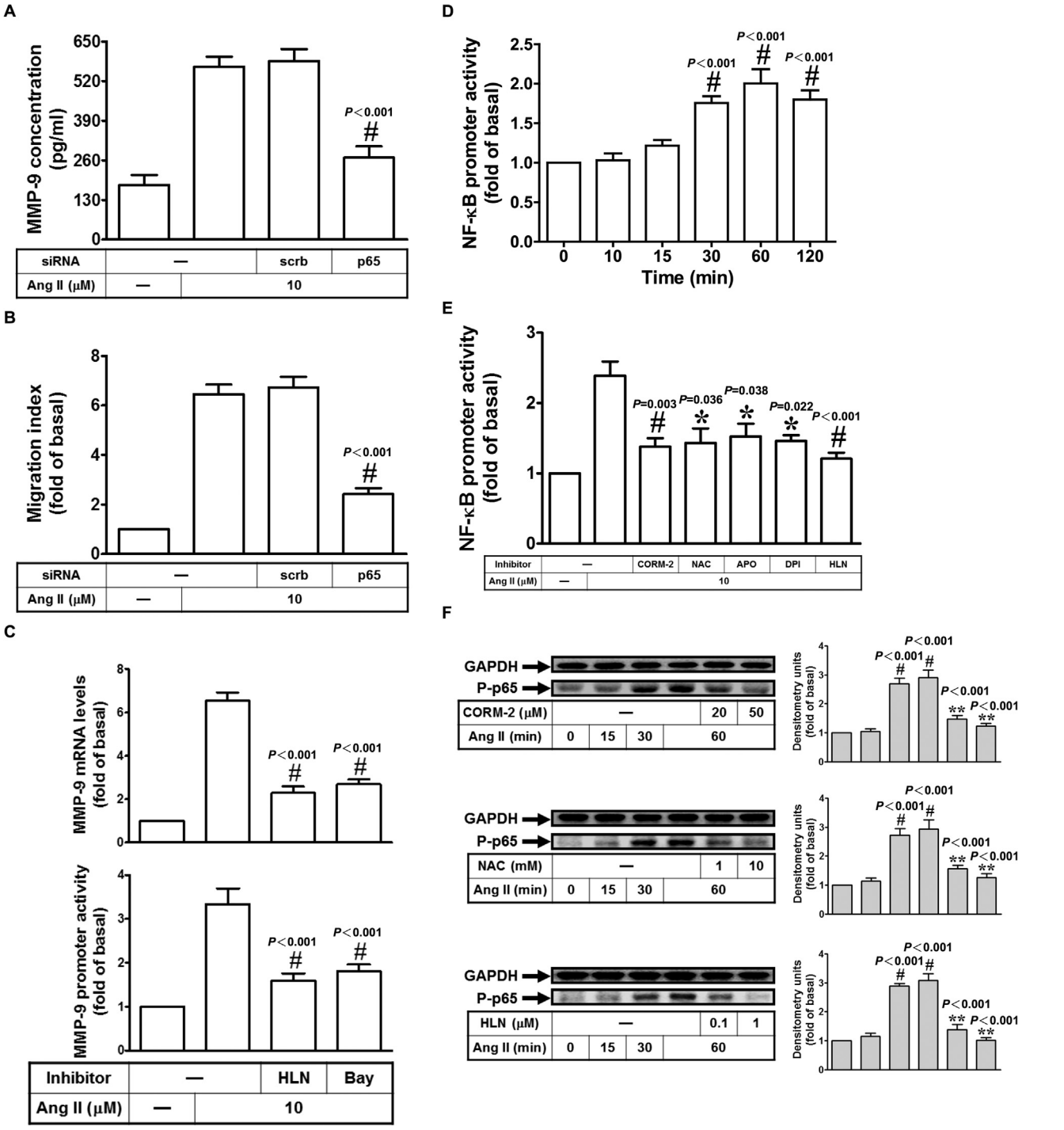

Fig. 5. *CORM-2 reduces Ang II-induced NF-κB activation in HASMCs.* Cells were transfected with siRNA of scrambled or p65, and then treated with Ang II for 24 h. The concentration of MMP-9 was determined (A). The cell migration was determined by migration assay (B). (C) Cells were pretreated with helenalin (HLN) or Bay11-7082 (Bay) for 1 h, and then treated with Ang II for 6 h. The mRNA levels and promoter activity of MMP-9 were determined by real-time PCR and promoter assay, respectively. (D) Cells were treated with 10 μM Ang II for the indicated times, and then the promoter activity of NF-κB was measured by promoter assay. (E) Cells were pretreated with CORM-2, NAC, APO, DPI, and HLN, and then treated with Ang II for 60 min. The promoter activity of NF-κB was measured by promoter assay. (F) Cells were pretreated with CORM-2, NAC, or HLN, and then treated with Ang II for the indicated times. The protein expression of phospho-p65 was determined. Data are expressed as mean ± S.E.M. of three independent experiments. #$P < 0.01$, as compared with the cells exposed to Ang II + scrambled siRNA (A, B). *$P < 0.05$; #$P < 0.01$, as compared with the cells exposed to Ang II alone (C, E). #$P < 0.01$, as compared with the basal level (D,F).**$P < 0.01$, as compared with the cells exposed to Ang II alone (F).

cardiovascular diseases, especially those involved vascular inflammation [54–56]. Several pathways were demonstrated to be involved in the anti-inflammatory effect of CORMs on VSMCs in various models of systemic or pulmonary vascular remodeling [54–58], which confirmed the protective role of CORMs through relaxation of vascular tone and reduction of VSMCs migration. In this paper we have described for the first time to our knowledge that CORM-2 can be used to reverse the pathological features of common cardiovascular disease. We found Ang II induced over-production of ROS was paralleled to NF-κB activation and the pro-inflammatory cytokine IL-6, which result in the activation of MMP-9 and migration of HASMCs. CORM-2 can exert anti-inflammatory effects through inhibition of NADPH oxidase [59,60].

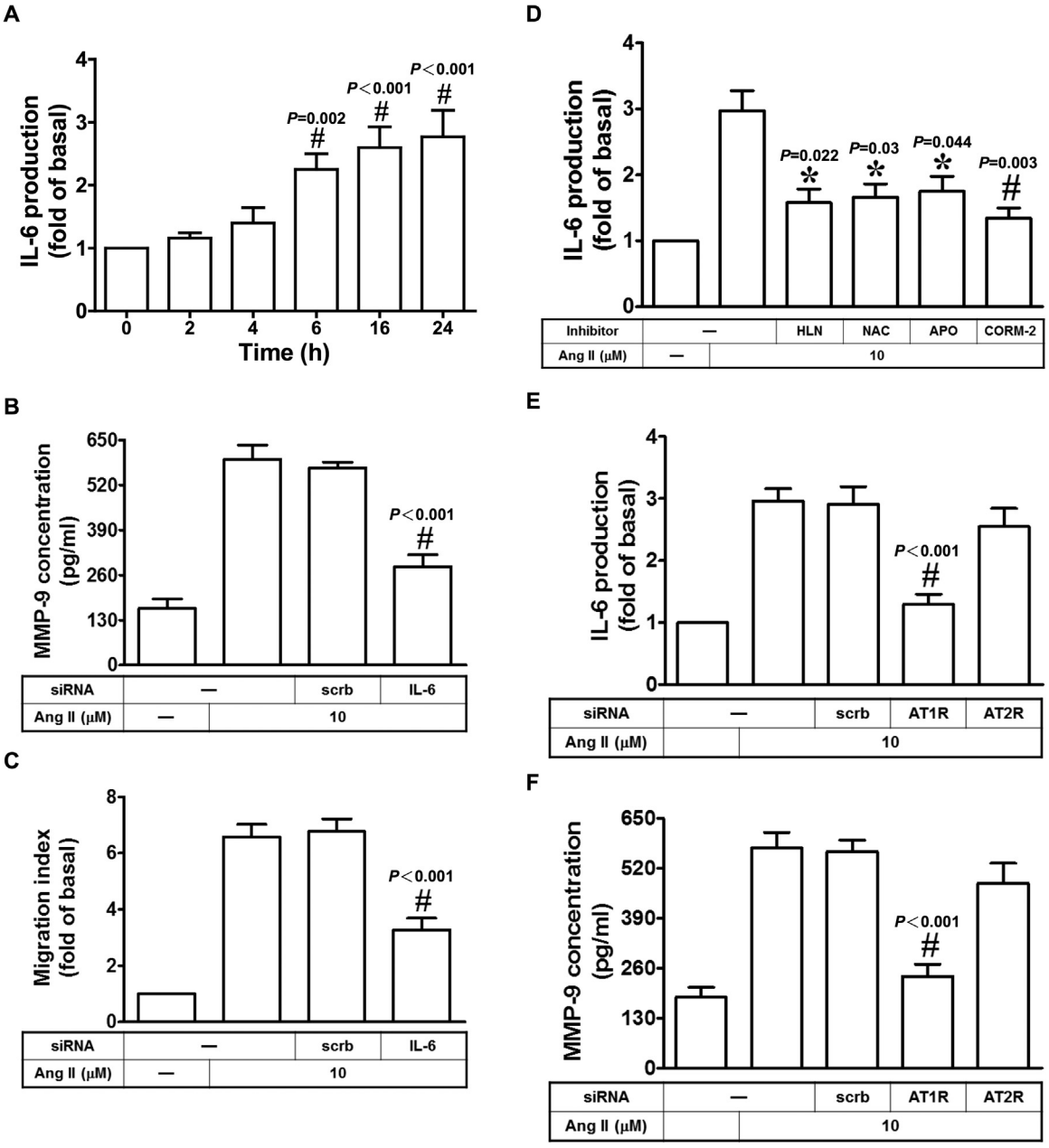

Fig. 6. *CORM-2 reduces Ang II-induced MMP-9 expression via inhibition of the AT1R/IL-6 pathway in HASMCs.* (A) Cells were treated with 10 μM Ang II for the indicated times, and then the production of IL-6 was measured. Cells were transfected with siRNA of scrambled or IL-6, and then treated with Ang II for 24 h. The concentration of MMP-9 was determined (B). The cell migration was determined by migration assay (C). (D) Cells were pretreated with HLN, NAC, APO, or CORM-2, and then treated with Ang II for 24 h. The production of IL-6 was measured. (E, F) Cells were transfected with siRNA of scrambled, AT1R, or AT2R, and then treated with Ang II for 24 h. The production of IL-6 and MMP-9 was measured. Data are expressed as mean ± S.E.M. of three independent experiments. #$P < 0.01$, as compared with the basal level (A). #$P < 0.01$, as compared with the cells exposed to Ang II + scrambled siRNA (B, C, E, F). *$P < 0.05$; #$P < 0.01$, as compared with the cells exposed to Ang II alone (D).

Therefore, the blockade of ROS generation by CORM-2 can be a preventive strategy of cardiovascular diseases.

NF-κB is the transcriptional factor that involves both pro- and anti-inflammatory roles [61]. Because NF-κB is extensively involved in the signaling pathways of various human diseases, there have been numerous studies toward development of inhibitors in this mediator [61,62]. In this report, we found NADPH oxidase/ROS have a significant regulatory role in NF-κB activation, which has been demonstrated in various cell types [63,64]. In addition, activation of NF-κB activity has been shown to induce MMP-9 expression in multiple types of human diseases and human cancer cells [63,65,66]. Hence, we examined whether NF-κB is involved in Ang II-induced upregulation of MMP-9 in HASMCs. Here we showed that Ang II induced NF-κB activation and enhanced NF-κB promoter activity in HASMCs, which could regulate MMP-9 expression and cell migration. Moreover, CORM-2 could inhibit Ang II-induced MMP-9 expression via inhibition of the activation of NF-κB in HASMCs.

Although NF-κB has been shown to induce expression of MMP-9, and then promotes cell migration, metastasis, and invasion [65–67], rare studies have investigated whether NF-κB directly binds to MMP-9 and controls the transcription of MMP-9 in these cells. Here we showed that the interaction between NF-κB and MMP-9 in HASMCs is mediated through IL-6 activation via IL-6 receptors. Increased IL-6 expression has been found to be associated with a variety of cardiovascular diseases, including atherosclerosis, obesity, myocardial infarction and type II diabetes [68,69]. IL-6 release can be stimulated by chronic inflammations, acute infections, physiological stress and sometimes obesity [69]. Furthermore, IL-6 is one of the most highly

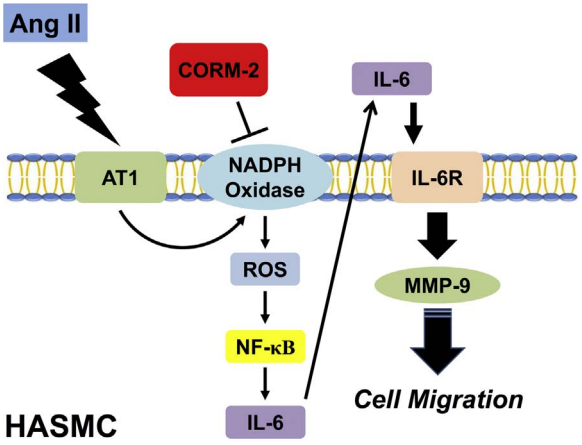

Fig. 7. Schematic diagram illustrating the proposed signaling pathway involved in Ang II-induced AT1R/IL-6/MMP-9-dependent cell migration. In HASMCs, Ang II induces MMP-9 expression via the AT1R/NADPH oxidase/ROS/NF-κB/IL-6 pathway. Moreover, CORM-2 can inhibit cell migration via inhibition of the activation of AT1R/NADPH oxidase/ROS/NF-κB/IL-6/IL-6R/MMP-9.

cytokines induced by NF-κB [70]. Although IL-6-induced expression of MMP-9 in macrophage has been demonstrated in the pathogenesis of chronic inflammation and cancer [71,72], the role of IL-6 linked to MMP-9 in cardiovascular diseases was rarely investigated. Indeed we found that Ang II markedly induced IL-6 release, which further caused MMP-9 induction and HASMCs migration. Moreover these responses induced by Ang II were mediated via AT1R, but not AT2R. CORM-2 can reduce MMP-9 expression via inhibition of IL-6 release in HASMCs.

Indeed, we also observed that Ang II induced MMP-2 concentration and the inhibition of MMP-2 also reduced Ang II-induced cell migration (Supplementary Fig. 1). Finally, we found that CORM-2 pretreatment could decrease Ang II-induced MMP-2 concentration. Thus, we suggest that MMP-2 also possibly plays a key role in regulating Ang II-induced cell migration. However, in this study, we focused on the signaling pathways involved in Ang II-induced COX-2/PGE2/IL-6/MMP-9 expression. In the future, we will investigate if these signaling pathways are also involved in Ang II-induced MMP-2 expression.

In summary, as depicted in Fig. 7, Ang II induced AT1R/NADPH oxidase/ROS/NF-κB/IL-6-dependent MMP-9 expression and migration of HASMCs. Moreover, pretreatment with CORM-2 inhibited Ang II-induced NADPH oxidase/ROS generation, NF-κB activation, IL-6 release, and MMP-9-dependent cell migration. Thus, as induction of exogenous CO may serve as a potential therapeutic target, continuous efforts toward identifying novel heart and blood vessels protective antioxidant/anti-inflammatory substances that targeting CO and establishment of well-designed *in vivo* models properly evaluating the efficacy of these agents will be warranted.

4. Materials and methods

4.1. Materials

Cell Signaling (Danver, MA) provided us the anti-phospho-p65 antibody. Helenalin (HLN), Bay11-7082 (Bay), diphenyleneiodonium (*DPI*), N-acetyl-L-cysteine (NAC), and apocynin (APO) were purchased from Biomol (Plymouth Meetings, PA). Anti-GAPDH, anti-GαS, and anti-MMP-9 antibodies were purchased from Santa Cruz (Santa Cruz, CA). We bought the Bicinchoninic acid (BCA) protein assay from Pierce (Rockford, IL). Tricarbonyldichlororuthenium (II) dimer (*CORM-2*), *hemoglobin (Hb),* enzymes, Ang II, and other chemicals were obtained from Sigma (St. Louis, MO).

4.2. Cell culture

HASMCs were obtained as cryopreserved tertiary cultures from Cascade Biologics (OR) and grown in culture flasks in smooth muscle cells growth medium M231 (Cascade Biologics, Inc.) This medium was supplemented with fetal bovine serum (FBS, 5%), human basic fibroblast growth factor (3 ng/ml), human epidermal growth factor (10 ng/ml), Fungizone (1.25 mg/ml), streptomycin (100 pg/ml), penicillin (100 U/ml), and insulin (10 mg/ml) in a humidified 5% CO_2 atmosphere at 37 °C. We changed the growth medium every other day until the cells became confluence, then we passed these cells, and cultured until the cells grew to confluence again. We used cells at between passages 3 and 7 in this study.

4.3. Transient transfection with siRNAs

We purchased the human siRNAs of scrambled, Nox2, MMP-9, $p47^{phox}$, p65, IL-6, Nox4, AT1R, and AT2R from Sigma (St. Louis, MO). Transient transfections with various siRNAs (100 nM) were performed using Lipofectamine transfection reagent in accordance with the manufacturer's protocols. The siRNA used in this study were labeled with EGFP. The transfection efficiency was evaluated by transfection with EGFP, and was determined to be approximately 60%.

4.4. Real-Time PCR

Total RNA of HASMCs was extracted using TRIzol reagent and reverse-transcribed into cDNA. We used real-time PCR and SYBR Green PCR reagents (Applied Biosystems, Branchburg, NJ, USA) and primers specific for MMP-9 and GAPDH mRNAs to analyze the reverse-transcribed cDNA from mRNA. We determined the levels of MMP-9 mRNA expression by normalizing to that of GAPDH expression.

4.5. Migration assay

HASMCs were cultured to confluence in 10-cm dishes and starved with serum-free M231 for 24 h. We used a blade to scratch the monolayer cells to create extended and clear scratches in the center of the dishes in order to make a bright and clear field. We washed the cells once with PBS to remove the detached cells. After preincubation with the various inhibitors for 1 h, we added serum-free M231 with or without Ang II to each dish which contained a DNA synthesis inhibitor hydroxyurea (10 μM). We obtained images of migratory cells from the scratched boundary at 0 and 24 h using a digital camera (Canon, Japan) and a light microscope (Olympus, Japan). We counted the migratory cells numbers from the resulting four phase images for each point and then made an average for each experimental condition. This experiment was repeated, and we generated the data from at least four independent assays.

4.6. Isolation of cell fractions

The cytosolic and membrane fractions were isolated according to the standard procedure [32]. Cells were lysed in lysis buffer, and then centrifuged at 16,000×*g* for 20 min at 4 °C. The supernatant which represented the cytosolic fraction was collected. Following centrifugation, the pellet (representing the membrane fraction) was collected.

4.7. Cell viability

The MTT assay was used for evaluation of cell viability. HASMCs were seeded onto 96-well plates overnight. After treatment with different concentrations of Ang II or CORM-2, MTT solution (5 mg/ml) was added. Following incubation for 2 h at 37 °C, the plates were read using a microplate spectrophotometer at a wavelength of 540 nm.

4.8. Measurement of MMP-9 luciferase activity

The human MMP-9 promoter activity was evaluated using the luciferase assay kit (Promega, Madison, WI, USA) as described previously [73]. The cells were transfected with the MMP-9-luc plasmid (−720 to −11 bp). Following treatment, luciferase assay solution was added, and the luminescence was determined using a luminometer. The promoter luciferase activities were standardized to β-galactosidase.

4.9. Gelatin zymography

Gelatin zymography is used to detect gelatinase activity, specially MMP-2 and -9. In this study, we seeded HASMCs onto 6-well culture plates and made them quiescent at confluence after incubation in serum-free M231 for 24 h. We used growth-arrested cells, which were incubated with Ang II for the indicated time intervals at 37 °C. We collected the culture medium and then the medium was centrifuged at 10,000×*g* for 5 min at 4 °C to remove cell debris. The MMP-9 expression was analyzed according to standard procedure, which has been described in our previous studies [74].

4.10. Western blot analysis

HASMCs were seeded onto 12-well plates and serum starved for 24 h. Following treatment with Ang II with or without inhibitors or siRNAs for different periods of time, cells were collected and lysed in lysis buffer. The protein concentration was measured by the BCA kit. Proteins were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Following transfer onto nitrocellulose membranes, the proteins were incubated with various antibodies overnight at 4 °C, including anti-MMP-9, anti-$p47^{phox}$, and anti-phospho-p65. Secondary anti-rabbit or anti-mouse horseradish peroxidase antibodies (1:2000 dilution) were then added for 1 h, and the bands were visualized using ECL reagents and developed by Hyperfilm-ECL.

4.11. RT-PCR analysis

Here, we isolated total RNA using TRIzol according to the protocol of the manufacturers' instructions. We obtained the cDNA from 0.5 μg total RNA and used it as a template for PCR amplification, which has been described in our previous study [32]. We used the primers in this study as the following:

GAPDH:

5′-ACCACAGTCCATGCCATCAC-3′ (sense).

5′-TCCACCACCCTGTTGCTGT-3′ (anti-sense).

AT1R:

5′-CCGAATTCACCATGTACCCATACGATGTTCCAGATTACGCTATTCTC-AACTCTTCTACTGAAG-3′ (sense).

5′-CCAGATCTCTACAGATCCTCTTCAGAGATGAGTTTCTGCTCGCGGCC-GCACTCAACCTCAAAACATGGTG-3′ (anti-sense).

AT2R:

5′-CCGAATTCACCATGGACTACAAAGACGATGACGACAAGAAGGGCAA-CTCCACCCTTGC-3′ (sense).

5′-CCAGATCTCTACAGATCCTCTTCAGAGATGAGTTTCTGCTCGCGGCC-GCAAGACACAAAGGTCTCCATTTC-3′ (anti-sense).

4.12. Measurement of MMP-9 and IL-6 generation

Cells were cultured in 6-well culture plates. After we found HASMCs became confluent, the cells were incubated with Ang II for the indicated time intervals. The media were collected and stored at −80 °C until next experiments. Moreover, we assayed the levels of MMP-9 and IL-6 using a MMP-9 ELISA kit (Thermo Scientific, Rockford, USA) and an IL-6 ELISA kit (BioSource International, Camarillo, CA), respectively, according to the manufacturer's instructions.

4.13. Measurement of intracellular ROS accumulation

We used the CellROX Green Reagent (Molecular Probes, Eugene, OR) to measure oxidative stress in the cells. Following treatment, 5 μM CellROX Green Reagent was added to cells for 30 min at 37 °C. The cells were then harvested and the fluorescence was determined using a Fluoroskan Ascent FLplatereader with wavelengths of 485 nm (excitation) and 520 nm (emission).

4.14. Determination of NADPH oxidase activity

The NADPH oxidase activity in these cells was evaluated by the lucigenin chemiluminescence assay as described previously [59]. Following treatment, the cell membrane fraction was collected, NADPH (Sigma) (1 μM) and lucigenin (Sigma) (20 μM) were added, and chemiluminescence was determined using a Fluoroskan Ascent FL (Thermo®) in an out-of-coincidence mode.

4.15. Statistical analysis of data

Data were showed as the mean or mean ± SEM of three independent experiments and estimated using GraphPad Prism Program (GraphPad, San Diego, CA, USA). All the data were analyzed by one-way ANOVA followed with Tukey's post-hoc test. A p value of less than 0.05 was considered significant.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Ms. Sin-Yu Li for their technical assistance. This work was supported by the Chang Gung Medical Research Foundation, grant number CMRPGMF0031, CMRPGMC0011, CMRPGMC0012, and CMRPGME0021; the Ministry of Science and Technology, Taiwan, grant number MOST 104-2320-B-039-043; the China Medical University, grant number CMU105-S-46, CMU104-N-05, and CMU105-N-22.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.redox.2017.02.019.

References

[1] B.S. van Thiel, I. van der Pluijm, L. te Riet, J. Essers, A.H. Danser, The renin-angiotensin system and its involvement in vascular disease, Eur. J. Pharmacol. 763 (Pt A) (2015) 3–14.

[2] A.R. da Silva, R.A. Fraga-Silva, N. Stergiopulos, F. Montecucco, F. Mach, Update on the role of angiotensin in the pathophysiology of coronary atherothrombosis, Eur. J. Clin. Invest. 45 (3) (2015) 274–287.

[3] C.M. Ferrario, W.B. Strawn, Role of the rennin-angiotensin-aldosterone system and proinflammatory mediators in cardiovascular disease, Am. J. Cardiol. 98 (2006) 121–128.

[4] A.J. Ferreira, M. Bader, R.A. Santos. Therapeutic targeting of the angiotensin-converting enzyme 2/Angiotensin-(1-7)/Mas cascade in the renin-angiotensin system: a patent review, 22, 2012, pp. 297–302.

[5] F. Jiang, J. Yang, Y. Zhang, M. Dong, S. Wang, Q. Zhang, et al., Angiotensin-converting enzyme 2 and angiotensin 1-7: novel therapeutic targets, Nat. Rev. Cardiol. 11 (7) (2014) 413–426.

[6] T. Watanabe, K. Sato, F. Itoh, Y. Noguchi, K. Fujimoto, T. Koyama, et al., Emerging roles for vasoactive peptides in diagnostic and therapeutic strategies against atherosclerotic cardiovascular diseases, Curr. Protein Pept. Sci. 14 (6) (2013) 472–480.

[7] M.L. McCormick, D. Gavrila, N.L. Weintraub, Role of oxidative stress in the pathogenesis of abdominal aortic aneurysms, Arterioscler. Thromb. Vasc. Biol. 27 (3) (2007) 461–469.

[8] S.H. Tsai, P.H. Huang, Y.J. Hsu, Y.J. Peny, C.H. Lee, J.C. Wang, et al., Inhibition of hypoxia inducible factor-1α attenuates abdominal aortic aneurysm progression through the down-regulation of matrix metalloproteinases, Sci. Rep. 6 (2016) 28612.

[9] A.N.D. Cat, A.C. Montezano, D. Burger, R.M. Touzy, Angiotensin II, NADPH oxidase, and redox signaling in the vasculature, Antioxid. Redox Signal. 19 (10) (2013) 1110–1120.

[10] T. Bruder-Nascimento, P. Chinnasamy, D.F. Riascos-Bernal, S.B. Cau, G.E. Callera, R.M. Touyz, et al., Angiotensin II induce Fat1 expression/activation and vascular smooth muscle cell migration via Nox 1-dependent reactive oxygen species generation, J. Mol. Cell. Cardiol. 66 (2014) 18–26.

[11] S.H. Chan, J.Y. Chan, Angiotensin-generated reactive oxygen species in brain and pathogenesis of cardiovascular diseases, Antioxid. Redox Signal. 19 (10) (2013) 1074–1084.

[12] A.C. Montezano, D.C.A. Nguyen, F.J. Rois, R.M. Touyz, Angiotensin II and vascular injury, Curr. Hyperens. Rep. 16 (6) (2014) 431.

[13] S.W. Ryter, J. Alam, A.M. Choi, Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications, Physiol. Rev. 86 (2) (2006) 583–650.

[14] S.W. Ryter, A.M. Choi, Heme oxygenase–1/carbon monoxide: from metabolism to molecular therapy, Am. J. Respir. Cell. Mol. Biol. 41 (3) (2009) 251–260.

[15] K. Nakahira, A.M. Choi, Carbon monoxide in the treatment of sepsis, Am. J. Physiol. Lung. Mol. Physiol. 309 (12) (2015) L1387–L1393.

[16] S.W. Ryter, A.M. Choi, Targeting heme oxygenase-1 and carbon monoxide for therapeutic modulation of inflammation, Transl. Res. 167 (1) (2016) 7–34.

[17] K. Katada, T. Takagi, K. Uchiyama, Y. Naito, Therapeutic roles of carbon monoxide in intestinal ischemia-reperfusion injury, J. Gastroenterol. Hepatol. 30 (Suppl 1) (2015) S46–S52.

[18] M.M. Al-Owais, J.L. Scragg, M.L. Dallas, H.E. Boycott, P. Warburton, A. Chakrabarty, J.P. Boyle, C. Peers, Carbon monoxide mediates the anti-apoptotic effects of heme oxygenase-1 in medulloblastoma DAOY cells via K^+ channel inhibition, J. Biol. Chem. 287 (2012) 24754–24764.

[19] N. Chiang, M. Shinohara, J. Dalli, V. Mirakaj, M. Kibi, A.M. Choi, C.N. Serhan, Inhaled carbon monoxide ceelerates resolution of inflammation via unique proresolving mediator-heme oxygenase-1 circuits, J. Immunol. 190 (12) (2013) 6378–6388.

[20] R. Motterlini, L.E. Otterbein, The therapeutic potential of carbon monoxide, Nat. Rev. Drug. Discov. 9 (9) (2010) 728–743.

[21] L.K. Warenham, R.K. Poole, M. Tinajero-Trejo, CO-releasing metal carbonyl compounds as antimicrobial agents in the post-antibiotic Era, J. Biol. Chem. 290 (31) (2015) 18999–19007.

[22] O.S. Qureshi, A. Zeb, M. Akram, M.S. Kim, J.H. Kang, H.S. Kim, et al., Enhanced acute anti-inflammatory effects of CORM-2-loaded nanoparticles via sustained carbon monoxide delivery, Eur. J. Pharm. Biopharm. 108 (2016) 187–195.

[23] S. Lian, Y. Xia, T.T. Ung, P.N. Khoi, H.J. Yoon, N.H. Kim, et al., Carbon monoxide releasing molecule-2 ameliorates IL-1β-induced IL-8 in human gastric cancer cells, Toxicology 12 (361–362) (2016) 24–38.

[24] M. Desmard, N. Amara, S. Lanone, R. Motterlini, J. Boczkowski, Carbon monoxide reduces the expression and activity of matrix metalloproteinases 1 and 2 in alveolar epithelial cells.

[25] A. Yabluchanskiy, Y. Ma, R.P. Iyer, M.E. Hall, M.L. Lindsey, Matrix metalloproteinase-9: many shades of function in cardiovascular disease, Physiology 28 (6) (2013) 391–403.

[26] J. Barallobre-Barreiro, A. Didangelos, F.A. Schoendube, I. Drozdov, X. Yin, M. Fernandez-Caggiano, et al., Proteomics analysis of cardiac extracellular matrix remodeling in a porcine model of ischemia/reperfusion injury, Circulation 125 (2012) 789–802.

[27] R.P. Iyer, L.E. de Castro, Bras, N.L. Patterson, M. Bhowmick, E.R. Flynn, M. Asher, et al., Early matrix metalloproteinase-9 inhibition post-myocardial infarction worsens cardiac dysfunction by delaying inflammation resolution, J. Mol. Cell. Cardiol. 100 (2016) 109–117.

[28] P. Prathipati, N. Metreveli, S.S. Nandi, S.C. Tyagi, P.K. Mishra, Ablation of matrix metalloproteinase-9 prevents cardiomyocytes contractile dysfunction diabetics, Front. Physiol. 7 (2016) 93.

[29] A. Dufour, S. Zucker, N.S. Sampson, C. Kuscu, J. Cao, Role of matrix metalloproteinase-9 dimers in cell migration: design of inhibitory peptides, J. Biol. Chem. 285 (2010) 35944–35956.

[30] A.I. Rodriguez, A. Gangopadhyay, E.E. Kelley, P.J. Pagano, B.S. Zuckerbraun, P.M. Bauer, HO-1, and CO decrease platelet-derived growth factor-induced vascular smooth muscle cell migration via inhibition of Nox1, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 98–104.

[31] H.J. Sun, M.X. Zhao, X.S. Ren, T.Y. Liu, Q. Chen, Y.H. Li, Y.M. Kang, J.J. Wang, G.Q. Zhu, Salusin-β promotes vascular smooth muscle cell migration and intimal hyperplasia after vascular injury via ROS/NF-κB/MMP-9 pathway, Antioxid. Redox Signal. 24 (2016) 1045–1057.

[32] I.T. Lee, S.W. Wang, C.W. Lee, C.C. Chang, C.C. Lin, S.F. Luo, C.M. Yang, Lipoteichoic acid induces HO-1 expression via the TLR2/MyD88/c-Src/NADPH oxidase pathway and Nrf2 in human tracheal smooth muscle cells, J. Immunol. 181 (2008) 5098–5110.

[33] G. Cepinskas, K. Katada, A. Bihari, R.F. Potter, Carbon monoxide liberated from carbon monoxide-releasing molecule CORM-2 attenuates inflammation in the liver of septic mice, Am. J. Physiol. Gastrointest. Liver Physiol. 294 (2008) G184–G191.

[34] T. Chen, W. Wang, J.R. Li, H.Z. Xu, Y.C. Peng, L.F. Fan, F. Yan, C. Gu, L. Wang, G. Chen, PARP inhibition attenuates early brain injury through NF-κB/MMP-9 pathway in a rat model of subarachnoid hemorrhage, Brain Res. 1644 (2016) 32–38.

[35] S. Sindhu, A. Al-Roub, M. Koshy, R. Thomas, R. Ahmad, Palmitate-induced MMP-9 expression in the human monocytic cells is mediated through the TLR4-MyD88 dependent mechanism, Cell. Physiol. Biochem. 39 (2016) 889–900.

[36] Z. Zhang, Q. Duan, H. Zhao, T. Liu, H. Wu, Q. Shen, C. Wang, T. Yin, Gemcitabine treatment promotes pancreatic cancer stemness through the Nox/ROS/NF-κB/STAT3 signaling cascade, Cancer Lett. 382 (2016) 53–63.

[37] B. Kim, H.S. Kim, S. Kim, G. Haegeman, B.K. Tsang, D.N. Dhanasekaran, Y.S. Song, Adipose stromal cells from visceral and subcutaneous fat facilitate migration of ovarian cancer cells via IL-6/JAK2/STAT3 pathway, Cancer Res. Treat. (2016) in press.

[38] S. Ren, Y. Zhou, X. Fang, X. She, Y. Wu, X. Wu, PRL-3 is involved in estrogen- and IL-6-induced migration of endometrial stromal cells from ectopic endometrium, Reprod. Sci. (2017) in press.

[39] Y.V. Gowrisankar, M.A. Clark, Angiotensin II induces interleukin-6 expression in astrocytes: role of reactive oxygen species and NF-κB, Mol. Cell. Endocrinol. 437 (2016) 130–141.

[40] A. Nguyen Dinh Cat, A.C. Montezano, D. Burger, R.M. Touyz, Angiotensin II, NADPH oxidase, and redox signaling in the vasculature, Antioxid. Redox Signal. 19 (2013) 1110–1120.

[41] C. Savoia, D. Burger, N. Nishigaki, A. Montezano, R.M. Touyz, Angiotensin II and the vascular phenotype in hypertension, Expert Rev. Mol. Med. 13 (2011) e11.

[42] E.R. Simo-Cheyou, J.J. Tan, R. Grygorczyk, A.K. Srivastava, STIM-1 and ORAI-1 channel mediate angiotensin-II-induced expression of Egr-1 in vascular smooth muscle cells, J. Cell. Physiol. 20 (2017) (Epub ahead of print).

[43] C. Li, R. Han, L. Kang, J. Wang, Y. Gao, Y. Li, et al., Pirfenidone controls the feedback loop of the AT1R/p38 MAPK/renin-angiotensin system axis by regulating liver X receptor-α in myocardial infarction-induced cardiac fibrosis, Sci. Rep. 7 (2017) 40523.

[44] A. Meissner, F. Miro, F. Jiménez-Altayó, A. Jurado, E. Villa, A.M. Planas, Sphingosine-1-phosphate signalling-a key player in the pathogenesis of Angiotensin II-induced hypertension, Cardiovasc. Res. 113 (2) (2017) 123–133.

[45] J. Han, C. Zou, L. Mei, Y. Zhang, Y. Qian, S. You, et al., MD2 mediates angiotensin II-induced cardiac inflammation and remodeling via directly binding to Ang II and activating TLR4/NF-kB sinaling pathway, Basic Res. Cardiol. 112 (1) (2017) 9.

[46] R.W. Guo, L.X. Yang, H. Wang, B. Liu, L. Wang, Angiotensin II induces matrix metalloproteinase-9 expression via a nuclear-kappa B-dependent pathway in vascular smooth muscle cells, Regul. Pept. 147 (1–3) (2008) 37–44.

[47] Y.V. Gowrisankar, M.A. Clark, Angiotensin II induces interleukin-6 expression in astrocytes: role of reactive oxygen species and NF-kB, Mol. Cell. Endocrinol. 437 (2016) 130–141.

[48] Y.M. Kang, D.M. Zhang, X.J. Yu, Q. Yang, J. Qi, Q. Su, et al., Chronic infusion of enalaprilat into hypothalamic paraventricular nucleus attenuates angiotensin II-induced hypertension and cardiac hypertrophy by restoring neurotransmitters and cytokines, Toxicol. Appl. Pharmacol. 274 (3) (2014) 436–444.

[49] M. Khan, F. Hassan, S. Roy, C.K. Sen, Measurement of reactive oxygen species in cardiovascular disease, Hypertension 49 (4) (2014) 359–370.

[50] P.R. Angelova, A.Y. Abramov, Functional role of mitochondrial reactive oxygen species in physiology, Free. Radic. Biol. Med. 16 (2016) 30293.

[51] G.R. Drummond, S. Selemidis, K.K. Griendling, C.G. Sobey, Combating oxidative stress in vascular disease: nadph oxidases as therapeutic targets, Nat. Rev. Drug. Discov. 10 (6) (2011) 453–471.

[52] H. Christou, T. Morita, C.M. Hsieh, H. Koike, B. Arkonac, M.A. Perrella, S. Kourembanas, Prevention of hypoxia-induced pulmonary hypertension by enhancement of endogenous heme oxygenase-1 in the rat, Circ. Res. 86 (12) (2000) 1224–1229.

[53] S. Schuhmacher, P. Wenzel, E. Schulz, M. Oelze, C. Mang, J. Kamuf, et al., Pentaerythritol tetranitrate improves angiotensin II-induced vascular dysfunction via induction of heme oxygenase-1, Hypertension 55 (4) (2010) 897–904.

[54] L.E. Fredenburgh, A.A. Merz, S. Cheng, Haeme oxygenase signaling pathway: implications for cardiovascular disease, Eur. Heart J. 36 (24) (2015) 1512–1518.

[55] S. Abid, A. Houssaïni, N. Mouraret, E. Marcos, V. Amsellem, F. Wan, et al., P21-dependent protective effects of a carbon monoxide-releasing molecule-2 in pulmonary hypertension, Arterioscler. Thromb. Vasc. Biol. 34 (2) (2014) 304–312.

[56] P. Failli, A. Vannacci, L. Di Cesare Mannelli, R. Motterlni, E. Masini, Relaxant effect of a water soluble carbon monoxide-releasing molecule (CORM-3) on spontaneously hypertensive rat aortas, Cardiovasc. Drugs Ther. 26 (4) (2012) 285–292.

[57] P. Wenzel, H. Rossmann, C. Müller, S. Kossmann, M. Oelez, A. Schulz, et al., Heme oxygenase-1 suppresses a pro-inflammatory phenotype in monocytes and determines endothelial function and arterial hypertension in mice and humans, Eur. Heart J. 36 (48) (2015) 3437–3446.

[58] Y. Wei, P. Chen, M. de Bruyn, W. Zhang, E. Bremer, W. Helfrich, Carbon monoxide-releasing molecules-2 (CORM-2) attenuates acute hepatic ischemia reperfusion injury in rats, Bmc. Gastroenterol. 10 (2010) 10.

[59] P.L. Chi, C.J. Liu, I.T. Lee, Y.W. Chen, L.D. Hsiao, C.M. Yang, HO-1 induction by CO-RM2 attenuates TNF-α-induced cytosoic phospholipase A2 expression via inhibition of PKCα-dependent NADPH oxidase/ROS and NF-κB, Mediat. Inflamm. 20 (2014) 279171.

[60] C. Taillé, J. El-Benna, S. Lanone, J. Boczkowski, R. Motterlini, Mitochondrial respiratory chain and NADPH oxidase are targets for the antiproliferative effect of carbon monoxide in human airway smooth muscle, J. Biol. Chem. 280 (27) (2005)

(25450-25360).
[61] T. Lawrence, The nuclear factor NF-kappaB pathway in inflammation, Cold. Spring Harb. Perspect. Biol. 1 (6) (2009) a001651.
[62] F.D. Herrington, R.J. Carmody, C.S. Goodyear, Modulation of NF-κB signaling as a therapeutic target in autoimmunity, J. Biomol. Screen. 21 (3) (2016) 223–242.
[63] M.J. Binker-Cosen, D. Richards, B. Oliver, H.Y. Gaisano, M.G. Binker, L.I. Cosen-Binker, Palmitic acid increases invasiveness of pancreatic cancer cells AsPC-1 through TLR4/ROS/NF-κB/MMP-9 signaling pathway, Biochem. Biophys. Res. Commun. 12 (2017) (Epub ahead of print).
[64] Z.J. He, F.Y. Zhu, S.S. Li, L. Zhong, H.Y. Tan, K. Wang, Inhibiting ROS-NF-κB-dependent autophagy enhanced brazilin-induced apoptosis in head and neck squamous cell carcinoma, Food Chem. Toxicol. 101 (2017) 55–66.
[65] Q. Li, L. Zhang, Y. Han, Z. Jiang, Q. Wang, Propofol reduces MMP2 expression by inhibiting NF-κB activity in human MDA-MB-231 cells, Biomed. Pharmacother. 66 (1) (2012) 52–56.
[66] S.J. Kao, J.L. Su, C.K. Chen, M.C. Yu, K.J. Bai, J.H. Chang, et al., Osthole inhibits the invasive ability of human lung adenocarcinoma cells via suppression of NF-κB-mediated matrix metalloproteinase-9 expression, Toxicol. Appl. Pharmacol. 261 (1) (2012) 105–115.
[67] A.S. Baldwin, Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB, J. Clin. Invest. 107 (3) (2001) 241–246.
[68] E. Kozarov, K. Huber, J. Wojta, Infection-associated biomarkers of inflammation in atherosclerosis, Curr. Pharm. Des. 21 (13) (2015) 1776–1782.
[69] J. Hartman, W.H. Frishman, Inflammation and atherosclerosis: a review of the role of interleukin-6 in the development of atherosclerosis and the potential for targeted drug therapy, Cardiol. Rev. 22 (3) (2014) 147–151.
[70] A.R. Brasier, The nuclear factor-kappaB-interleukin-6 signaling pathway mediating vascular inflammation, Cardiovasc. Res. 86 (2) (2010) 211–218.
[71] K. Kessenbrock, V. Plaks, Z. Werb, Matrix metalloproteinases: regulators of the tumor microenvironment, Cell 141 (1) (2010) 52–67.
[72] P. Kothari, R. Pestana, R. Mesraoua, R. Elchaki, K.M. Khan, A.J. Dannenberg, D.J. Falcone, IL-6-mediated induction of matrix metalloproteinase-9 is modulated by JAK-dependent IL-10 expression in macrophages, J. Immunol. 192 (2014) 349–357.
[73] I.T. Lee, C.C. Lin, Y.C. Wu, C.M. Yang, TNF-α induces matrix metalloproteinase-9 expression in A549 cells: role of TNFR1/TRAF2/PKCα-dependent signaling pathways, J. Cell. Physiol. 224 (2010) 454–464.
[74] C.W. Lee, C.C. Lin, W.N. Lin, K.C. Liang, S.F. Luo, C.B. Wu, S.W. Wang, C.M. Yang, TNF-α induces MMP-9 expression via activation of Src/EGFR, PDGFR/PI3K/Akt cascade and promotion of NF-κB/p300 binding in human tracheal smooth muscle cells, Am. J. Physiol. Lung Cell. Mol. Physiol. 292 (2007) L799–L812.