Carbon monoxide activation of delayed rectifier potassium currents of human cardiac fibroblasts through diverse pathways

Hyemi Bae¹, Taeho Kim², and Inja Lim¹,*

¹Department of Physiology, College of Medicine, Chung-Ang University, Seoul 06974, ²Department of Internal Medicine, College of Medicine, Chung-Ang University Hospital, Seoul 06973, Korea

ABSTRACT To identify the effect and mechanism of carbon monoxide (CO) on delayed rectifier K⁺ currents (Iₖ) of human cardiac fibroblasts (HCFs), we used the whole-cell mode patch-clamp technique. Application of CO delivered by carbon monoxide-releasing molecule-3 (CORM3) increased the amplitude of outward K⁺ currents, and diphenyl phosphine oxide-1 (a specific Iₖ blocker) inhibited the currents. CORM3-induced augmentation was blocked by pretreatment with nitric oxide synthase blockers (L-NG-monomethyl arginine citrate and L-NG-nitro arginine methyl ester). Pretreatment with KT5823 (a protein kinase G blocker), 1H-[1,2,4] oxadiazolo-[4,3-a] quinoxalin-1-on (ODQ, a soluble guanylate cyclase blocker), KT5720 (a protein kinase A blocker), and SQ22536 (an adenylate cyclase blocker) blocked the CORM3 stimulating effect on Iₖ. In addition, pretreatment with SB239063 (a p38 mitogen-activated protein kinase [MAPK] blocker) and PD98059 (a p44/p42 MAPK blocker) also blocked the CORM3’s effect on the currents. When testing the involvement of S-nitrosylation, pretreatment of N-ethylmaleimide (a thiol-alkylating reagent) blocked CO-induced Iₖ activation and DL-dithiothreitol (a reducing agent) reversed this effect. Pretreatment with 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)-21H,23H porphyrin manganese (III) pentachloride and manganese (III) tetrakis (4-benzoic acid) porphyrin chloride (superoxide dismutase mimetics), diphenyleneiodonium chloride (an NADPH oxidase blocker), or allopurinol (a xanthine oxidase blocker) also inhibited CO-induced Iₖ activation. These results suggest that CO enhances Iₖ in HCFs through the nitric oxide, phosphorylation by protein kinase G, protein kinase A, and MAPK, S-nitrosylation and reduction/oxidation (redox) signaling pathways.

INTRODUCTION

Carbon monoxide (CO) is a harmful substance that is a common cause of morbidity and mortality from poisoning [1]. However, CO is also endogenously synthesized upon the heme degradation by heme oxygenases (HOs). Atrial and ventricular cardiac myocytes constitutively express HO-2, and inducible HO-1 is increased by various stress factors [2], including myocardial infarction [3]. In addition, HOs prevent myocardial infarction [4], heart failure [5], and ischemia-reperfusion injury [4,6].

CO is an important cellular messenger that is considered to be both a physiological signaling molecule like nitric oxide (NO) and a potential therapeutic [7]. CO is being studied as an inhalation therapy with numerous potential clinical applications [7]. CO shows positive effects on the cardiovascular system, including vasodilatory effects [8,9], anti-apoptotic activity [10,11], and immune modulation effects [12]. These properties may be executed in conjunction with NO [13].

Recently, ion channels have been recognized as important effectors in CO activities. CO has a wide range of ion channel tar-
gets, including K+ channels, that play key roles in its beneficial effects. K+ channels play a critical role in cardiac electrophysiology, and their dysfunction is linked to intracellular signaling, metabolism, remodeling, and arrhythmogenesis in many cardiovascular diseases [14]. Voltage-dependent K+ channels (VDKCs) regulate resting membrane potential, proliferation, and contractile responses in the heart. There are two types of VDKCs: delayed rectifier K+(Kv) channels and large-conductance Ca2+-activated K+(BK) channels. Each K+ channel type has distinct kinetics and regulation.

Cardiac Kv channels conduct K+ currents during the plateau phase of action potentials and play pivotal roles in cardiac repolarization, cardiac physiology, and pathophysiology. Disruption of normal Kv channel functions renders the heart susceptible to abnormal electrical activity and predisposes to arrhythmia. Inherited mutations or drug blockage of Kv channels can cause cardiac arrhythmias [15].

CO activates the BK channel [16] and TREK1 channel [17] and inhibits the Kv2.1 channel [11] and the inward rectifier K+ channel [18]. CO also induces both activation and inhibition of the epithelial Na’ channel [19,20] and L-type Ca2+ channel [21-23]. CO modulates these proteins through a variety of different mechanisms, although the precise mechanism by which CO differentially regulates each of these ion channels remains controversial.

Human cardiac fibroblasts (HCFs) are the most abundant cell type in the heart, making up about two-thirds of the cardiac cellular population [24,25]. Although cardiac myocytes occupy approximately 75% of normal myocardial tissue volume, they only account for 30%–40% of cell numbers [26]. Therefore, HCFs are crucial for maintaining the cardiac extracellular matrix [27] and electro-mechanical function in healthy and diseased myocardium [24,28,29]. These cells are capable of synchronizing the electrical activity of multicellular cardiac tissue over extended distances through electrotonic interactions. This synchronization is accompanied by extremely large local conduction delays, which might contribute to arrhythmia generation in fibrotic hearts [30].

HCFs are non-excitable cells, however, they have multiple ion currents whose distribution and properties are distinct from cardiomyocytes [31-33]. They can affect cardiomyocyte electrical activity [30] and induce arrhythmogenesis [34]. The direct electrical coupling between fibroblasts and ventricular cardiomyocytes has been demonstrated in co-culture conditions and the whole heart [17,24,30] via connexin-based gap junctions [35] and indirectly via the extracellular matrix and the release of soluble mediators [24].

Kv and BK channels are the main K+ channels in HCFs [32,36]. CO activates the BK channels of HCFs through protein kinase G (PKG), protein kinase A (PKA), and S-nitrosylation [37], but the CO effect and related mechanism for the Kv channels of HCFs are still unclear. We identified the effect of CO on Kv channels of HCFs and the involved signaling pathways using the whole-cell mode patch clamp technique.

**METHODS**

**Cell culture and reagents**

We used commercial human adult ventricular cardiac fibroblasts (HCF-av, Cat #6310; ScienCell, San Diego, CA, USA). Cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM; Welgene, Gyeongsan, Korea) with fetal bovine serum (10%; Welgene) and penicillin-streptomycin solution (100×; GenDEPOT, Barker, TX, USA) in an incubator with a humidified atmosphere of 5% CO2 and 95% air at 37°C. Confluent fibroblasts were detached by incubation with trypsin (0.25%; Welgene) and ethylene diamine tetraacetic acid (0.02%) in DMEM for several minutes. The detached cells were pelleted by centrifugation and then the supernatant was removed. The pellet was suspended in 1 mL of bath solution and the cells used in this study. Only cells in early passages (P4 to P7) were used to limit possible culture variation. Passage (P) is the number of times the cells are processed with trypsin and transferred to another flask.

**Electrophysiological recordings**

The Axopatch 200B Patch Clamp Amplifier (Axon Instruments, Foster City, CA, USA) was used for whole-cell mode patch clamping to record K+ currents from single HCFs. The K+ currents were filtered at 2 kHz and digitized at 10 kHz. pCLAMP 9.0 software (Axon Instruments) was used for data acquisition and analysis of the whole-cell currents. The recording patch pipettes were prepared from filament-containing borosilicate tubes (TW150F-4; World Precision Instruments, Sarasota, FL, USA) using a two-stage microelectrode puller (PC-10; Narishige, Tokyo, Japan) and were fire-polished using a microforge (MF-830; Narishige). The pipetted material exhibited a resistance of 2–3 MΩ. All electrophysiological experiments were carried out at room temperature. The bath solution to record delayed rectifier K+ currents (I_{Kd}) contained 150 mM NaCl, 5.4 mM KCl, 1 mM CaCl₂, 2 mM MgCl₂, 10 mM glucose, and 5 mM HEPES (pH adjusted to 7.35 with NaOH). The pipette solution contained 130 mM KCl, 1 mM CaCl₂, 2 mM MgCl₂, 10 mM HEPES, 10 mM EGTA, and 2 mM Mg-ATP (pH adjusted to 7.3 with KOH). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). To record only the I_{Ks} of the cells, 100 nM iberiotoxin (a specific large-conductance calcium-activated K+ channel blocker) was added to the bath solution and 10 mM EGTA was added to the pipette solution during the experiments.

**Statistical analysis**

SPSS version 22.0 software (IBM Corp., Armonk, NY, USA)
was used for statistical analysis. The paired Student’s t-test was used to evaluate differences between the means of two groups, whereas one-way analysis of variance was used for multiple groups. p-values < 0.05 were considered statistically significant. The results are presented as the mean ± standard error of the mean (SEM).

RESULTS

CORM3 activated $I_K$ in HCFs

We recorded macroscopic outward $K^+$ currents of HCFs using whole-cell mode patch clamping with a voltage protocol that consisted of depolarizing steps (from −80 mV to +50 mV) in 10 mV increments for 400 ms with −80 mV of holding potential. The recorded $K^+$ currents of the HCFs showed behavior typical of $I_K$: voltage dependency, fast activating but not inactivating properties to depolarization, and maintenance throughout the test pulse (Fig. 1). The $K^+$ currents were blocked by 1 µM diphenyl phosphine oxide-1 (DPO-1), a specific blocker of the Kv1.5 channel and ultra-rapid type $I_K$ (−94.5 ± 0.2% of control, at +30 mV, n = 7, Fig. 1A, B).

CORM3 (10 µM, a CO donor) significantly increased the $I_K$ amplitude (+56.2 ± 3.1% of control, at +30 mV, n = 7, Fig. 1A). iCORM3 (the inactive form of CORM3) had no significant effect on the currents (10 µM, +1.2 ± 9.9% of control, at +30 mV, n = 7, Fig. 1B). In current–voltage ($I$–$V$) curves (Fig. 1C), the currents were activated from −20 mV and also did not show strong outward rectification, a characteristic of large-conductance $Ca^{2+}$-activated $K^+$ currents (IBK). IBK is the other main $K^+$ current of HCFs.

![Fig. 1. Carbon monoxide (CO) increases delayed rectifier $K^+$ currents ($I_K$) of human cardiac fibroblasts (HCFs). Original recordings of the $K^+$ currents were obtained by repeated voltage step depolarization from −80 to +50 mV for 400 ms duration (holding potential was −80 mV) by whole-cell mode patch clamp recordings. (A) Representative outward $K^+$ currents show the changes before (control) and after application of (A) carbon monoxide-releasing molecule-3 (CORM3, a CO donor, 10 µM) or (B) iCORM3 (inactive CORM3, 10 µM). The currents were blocked by diphenyl phosphine oxide-1 (DPO-1, a blocker of the delayed rectifier $K^+$ channel and the Kv1.5 channel, 1 µM). (C) Summarized current–voltage ($I$–$V$) curves of steady-state currents for the effects of CORM3, iCORM3, and DPO-1. (D) Bar graphs showing the current density changes of the $K^+$ currents at +30 mV regarding the effects of CORM3, iCORM3, and DPO-1 (n = 7, each). (E) Concentration-dependent activation curve of $I_K$ by CORM3. The solid line shows the fit based on a standard dose-response relationship, which yielded an estimated half maximal effective concentration (EC50) of 11.38 µM for CORM3. ]
We added iberiotoxin (100 nM, the specific BK channel blocker) to the bath solution and 10 mM EGTA to the pipette solution to block \( I_{\text{BK}} \) \((p < 0.05, **p < 0.01, ***p < 0.001)\). CORM3 increased \( I_{\text{K}} \) current densities \((41.5 \pm 14.5 \text{ pA/pF at control, 64.8} \pm 4.4 \text{ pA/pF at CORM3, 2.28} \pm 0.27 \text{ pA/pF at DPO-1,} +30 \text{ mV, n = 7, } *p < 0.05, ***p < 0.001\)). CORM3’s concentration-response relationship showed that steady-state currents normalized by control data were fitted with the Hill equation, producing an estimated half-maximal excitation concentration (EC\(_{50}\)) value of 11.38 µM and a Hill coefficient of 0.7 (n = 7, Fig. 1E).

### Nitric oxide synthase (NOS)/NO involvement in CO–induced \( I_{\text{K}} \) activation of HCFs

To investigate the regulation of \( I_{\text{K}} \) by CO, we first explored the involvement of NO because CO is known to activate NOS and soluble guanylate cyclase (sGC) [22]. The CORM3-induced \( I_{\text{K}} \) activation was significantly suppressed by pretreatment with a NOS inhibitor, L-N\(^{4}\)-monomethyl arginine citrate (L-NMMA, 100 µM) for 20 min \((-10.2 \pm 9.3\% \text{ of control, n = 7, Fig. 2A, B).} \)

Pretreatment of cells with other NOS blocker, L-N\(^{4}\)-nitroarginine methyl ester (L-NAME, 100 µM) also attenuated the CORM3-induced \( I_{\text{K}} \) effect \((+4.0 \pm 11.7\% \text{ of control, n = 7, Fig. 2D, E).} \) L-NMMA or L-NAME itself did not affect \( I_{\text{K}} \) amplitude. The current densities at +30 mV of the CORM3 effects after L-NMMA or L-NAME pretreatment did not change (n = 7 each, Fig. 2C, F), suggesting that CO could activate \( I_{\text{K}} \) through NO formation by NOS.

### cGMP and cAMP signaling pathway involvement in CO-induced \( I_{\text{K}} \) activation of HCFs

We tested the stimulating effect of CORM3 on \( I_{\text{K}} \) was mediated by the cGMP signaling pathway. Under pretreatment by KTS823 (a PKG inhibitor, 1 µM), 10 µM CORM3 failed to increase \( I_{\text{K}} \) \((+1.3 \pm 11.3\% \text{ of the control, n = 7, Fig. 3A).} \) With ODQ (a soluble guanylate cyclase inhibitor, 10 µM) pretreatment, CORM3 could not increase \( I_{\text{K}} \) \((-4.9 \pm 8.8\% \text{ of the control, n = 7, Fig. 3B).} \) The current densities did not change significantly (n = 7, each, Fig. 3C).

We also tested whether the cAMP signaling pathway is in-

---

**Fig. 2. Nitric oxide synthase (NOS) blockers inhibits carbon monoxide (CO)–induced activation of delayed rectifier K\(^{+}\) currents (\( I_{\text{K}} \)) in human cardiac fibroblasts (HCFs).** Currents are obtained in which cells were repeatedly depolarized from –80 mV to +50 mV (400 ms duration). Carbon monoxide-releasing molecule-3 (CORM3) was applied either to untreated cells or to cells pretreated for 20 min with NOS blockers. (A) Representative original recordings of \( I_{\text{K}} \) show the CORM3 (10 µM) effect after pretreatment of L-N\(^{4}\)-monomethyl arginine citrate (L-NMMA, 100 µM) for 20 min. (B) Summarized current–voltage (I–V) relationship curves for \( I_{\text{K}} \) and (C) bar graphs for the summary of current density changes of \( I_{\text{K}} \) (at +30 mV) also show the effect of CORM3 (10 µM) on \( I_{\text{K}} \) after pretreatment of L-NMMA (100 µM) for 20 min. (D) Representative original recordings of \( I_{\text{K}} \) after pretreatment of L-NMMA (100 µM). (E) Summarized I–V relationship curves of \( I_{\text{K}} \) and (F) bar graphs for the summary of current density changes of \( I_{\text{K}} \) (at +30 mV) also show the CORM3 (10 µM) effect on \( I_{\text{K}} \) after L-N\(^{4}\)-nitroarginine methyl ester (L-NAME, 100 µM) pretreatment (n = 7 each). Values are mean ± SEM (n = 7 each).
CO activation of delayed rectifier K⁺ currents through diverse pathways

Involved in CO’s effect of CO on Iₖ in cells. A KT5720 (a PKA inhibitor, 1 µM) pretreatment for 20 min inhibited CORM3’s stimulation effect of on Iₖ (–1.8 ± 9.1% of the control, n = 7, Fig. 3D). A SQ22536 (an adenylate cyclase inhibitor, 100 µM) pretreatment also inhibited the CORM3 effect (–5.6 ± 12.9% of the control, n = 7, Fig. 3E). There were no significant changes in current density by CORM3 after KT5720 or SQ22536 pretreatment (Fig. 3F). These results suggest that both cGMP and cAMP signaling pathways are involved in CO’s effects on Iₖ in HCFs.

S-nitrosylation involvement in CO-induced Iₖ activation of HCFs

We examined whether CO activated Iₖ via direct S-nitrosylation of the target proteins' thiol residues. An alternative pathway for NO’s biological effects because CO activates Iₖ through this mechanism [37]. With N-ethylmaleimide (NEM, a thiol-alkylating reagent, 0.5 mM) pretreatment for 20 min, CORM3 failed to increase Iₖ (–1.28 ± 10.1% of the control, n = 7, Fig. 4A, B). The current densities of the Iₖ were unchanged by CORM3 after NEM treatment (Fig. 4C), which suggests that the ultimate target of CO is a thiol residue. NEM alone did not increase the currents. DL-dithiothreitol (DTT, a reducing agent, 5 mM) reversed CORM3-induced enhancement of Iₖ (CORM3, +68.6 ± 25.6% of the control; *p < 0.05 vs. control; DTT, +14.7 ± 13.9% of control, n = 7, **p < 0.05 vs. CORM3, Fig. 4D, E). The current densities of the Iₖ were significantly increased by CORM3 (42.5 ± 3.5 pA/pF at control, 71.6 ± 8.9 pA/pF at CORM3, n = 7, *p < 0.05) and reversed by DTT significantly (48.7 ± 4.8 pA/pF, n = 7, **p < 0.05 vs. CORM3, Fig. 4F). We conclude that CO also activates Iₖ of HCFs through S-nitrosylation.

MAPK signaling pathways involvement in CO-induced Iₖ activation of HCFs

We also tested the mitogen-activated protein kinase (MAPK) signaling pathway for the CO-induced activation of Iₖ because MAPKs are believed to be involved in the regulation of Kv channels [38]. Pretreatment with SB239063 (a p38 MAPK inhibitor, 10 µM) inhibited the CORM3 effect on Iₖ (–11.9 ± 7.7% of the control, n = 7, Fig. 5A). Pretreatment with PD98059 (a p44/p42 MAPK inhibitor, 10 µM) also inhibited CO-induced Iₖ activation (–3.7 ±
Redox signaling pathway involvement in CO-induced IK activation of HCFs

To explore the source of reactive oxygen species (ROS) involved in CO-mediated activation of IK of HCFs, cells were pretreated with two superoxide dismutase (SOD) mimetics, 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)-21H,23H porphyrin manganese (III) pentachloride (MnTMPyP, 50 μM) and manganese (III) tetras (4-benzoic acid) porphyrin chloride (MnTBAP, 10 μM). Both inhibited CO-induced IK activation (–9.3 ± 10.9% of the control, n = 7, Fig. 5D; +0.4 ± 13.1% of the control, n = 7, Fig. 5E). MnTMPyP (50 μM) and MnTBAP (10 μM) did not increase IK of HCFs (Fig. 5F). 

Fig. 4. S-nitrosylation involves carbon monoxide (CO)–induced activation of delayed rectifier K+ currents (IK) in human cardiac fibroblasts (HCFs). (A) Original recordings for the effect of carbon monoxide-releasing molecule-3 (CORM3, 10 μM) on IK after pretreatment with N-ethylmaleimide (NEM, a thiol-alkylating reagent, 0.5 mM) at +30 mV stimulation. (B) Summarized current–voltage (I–V) curves for the effect of CORM3 (10 μM) on IK after pretreatment with NEM (0.5 mM). (C) Bar graphs showing the current density changes for the CORM3 effects on IK after pretreatment with NEM (at +30 mV, n = 7 each). (D–F) The reversing effect of DL-dithiothreitol (DTT, a reducing agent, 5 mM) for CORM3-induced activation on IK (*p < 0.05 compared to control, #p < 0.05 compared to CORM3, n = 7 each).

The major findings of the present study are CO activates IK of cultured HCFs. NO through NOS, phosphorylation by PKG, PKA, and MAPKs, S-nitrosylation and reduction/oxidation (redox) signaling are involved in the CO-induced activation of the IK effect.

FIG. 4. S-nitrosylation involves carbon monoxide (CO)–induced activation of delayed rectifier K+ currents (IK) in human cardiac fibroblasts (HCFs). (A) Original recordings for the effect of carbon monoxide-releasing molecule-3 (CORM3, 10 μM) on IK after pretreatment with N-ethylmaleimide (NEM, a thiol-alkylating reagent, 0.5 mM) at +30 mV stimulation. (B) Summarized current–voltage (I–V) curves for the effect of CORM3 (10 μM) on IK after pretreatment with NEM (0.5 mM). (C) Bar graphs showing the current density changes for the CORM3 effects on IK after pretreatment with NEM (at +30 mV, n = 7 each). (D–F) The reversing effect of DL-dithiothreitol (DTT, a reducing agent, 5 mM) for CORM3-induced activation on IK (*p < 0.05 compared to control, #p < 0.05 compared to CORM3, n = 7 each).

4.8% of the control, n = 7, Fig. 5B). SB239063 (10 µM) or PD98059 (10 µM) did not increase the current density of IK of HCFs (Fig. 5C). These results suggest that the MAPK signaling pathway is also involved in the CO-induced activation of the IK effect.

Redox signaling pathway involvement in CO-induced IK activation of HCFs

To explore the source of reactive oxygen species (ROS) involved in CO-mediated activation of IK of HCFs, cells were pretreated with two superoxide dismutase (SOD) mimetics, 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)-21H,23H porphyrin manganese (III) pentachloride (MnTMPyP, 50 μM) and manganese (III) tetrais (4-benzoic acid) porphyrin chloride (MnTBAP, 10 μM). Both inhibited CO-induced IK activation (–9.3 ± 10.9% of the control, n = 7, Fig. 5D; +0.4 ± 13.1% of the control, n = 7, Fig. 5E). MnTMPyP (50 μM) and MnTBAP (10 μM) did not increase IK of HCFs (Fig. 5F). Diphenylene iodonium (DPI; 3 µM, an inhibitor of NADPH oxidases) or allopurinol (1 µM, a xanthine oxidase inhibitor) inhibited the CORM3-induced activation on IK (–7.5 ± 10.9% of the control, n = 7, Fig. 5G; –5.9 ± 10.8% of the control, n = 7, Fig. 5H). These results strongly suggest that ROS contributes to CO-mediated activation of IK of HCFs.

DISCUSSION

The major findings of the present study are CO activates IK of cultured HCFs. NO through NOS, phosphorylation by PKG, PKA, and MAPKs, S-nitrosylation and reduction/oxidation (redox) signaling are involved in the CO-induced activation of IK.

Identification of Kv channels in HCFs

In our experiments, the recorded outward K+ currents of HCFs in 100 nM iberiotoxin (a specific BK channel blocker) in the bath solution and 10 mM EGTA in the pipette solution showed typical characteristics of the IK (Fig. 1): rapid activation and no inactivation in kinetics, voltage dependency, and significant inhibition by DPO-1, a specific blocker for ultra-rapid type of IK (IKur) and hKv1.5 currents [39].

Kv channels include the slow, rapid, and ultra-rapid delayed rectifiers (IKs, IKr, and IKur) [14]. A functionally significant Kv channel with components corresponding to IKr and IKs is present...
in human ventricular cells, whereas $I_{Kur}$ is only present in atrial myocytes [40,41]. These findings explain the molecular, physiological, and pharmacological determinants of human atrial and ventricular repolarization and arrhythmias [42].

Kv1.5 is a rapidly activating, voltage-gated K$^+$ channel encoded by KCNA5 that inactivates slowly and incompletely [43,44]. Kv1.5 also contributes to repolarization of vascular smooth muscle cell membrane potential, limiting Ca$^{2+}$ entry and vascular tone. It is essential for balancing coronary blood flow with the metabolic demands of the working myocardium [45]. This channel is thought to be the major contributor to the $I_K$ in the human heart [46,47]. Its expression is largely confined to the atria and generates the $I_{Kur}$, the major repolarizing current that is active throughout phases 1–3 of the atrial action potential [48,49], and determines the duration and membrane potential of the plateau phase [41]. Because $I_{Kur}$ is atrium specific, $K_{1.5}$ channel is promising target as new, safer antiarrhythmic drugs to prevent atrial fibrillation without the risk of inducing QT prolongation and...
ventricular arrhythmias. DPO-1 also increases action potential duration in atrial but not ventricular myocytes and prevents atrial arrhythmias [50].

However, the strong mRNA expression of Kv1.5 for the Kv channels is also in HCFs [32,51] and ventricular cardiomyocytes [52]. These findings may indicate a functional role of these ion channel subunits in action potential formation in the human atrium and ventricle. This channel is also highly expressed in most vascular smooth muscle cells and is important for regulating cell excitability and maintaining basal tone.

HCFs make up the largest cell population in the heart and have cell–cell communication with cardiomyocytes and other cells [27]. The cardiomyocyte–cardiac fibroblast interactions are important in normal heart function and the development of diseases such as cardiac arrhythmia and fibrosis [35]. Kv channels may be useful therapeutic targets for cardiovascular disease.

**Effect of CO on $I_{K}$ in HCFs**

To test the CO effect on $I_{K}$, CORM3 was used as a CO donor because suitable delivery systems were required. CORMs are low-molecular weight compounds that release one or more CO molecules upon decomposition [9]. We achieved a reliable result for CO with CORM3 on $I_{K}$ of HCFs [37].

In this experiment, CO stimulated $I_{K}$ in HCFs concentration-dependently (Fig. 1). This is the first report that CO induces activation for $I_{K}$, to the best of our knowledge. In previous reports, CO inhibited $I_{K}$ in ventricular cardiomyocytes, native Kv1.5 recorded in HL-1 murine atrial cells [53], and recombinant Kv2.1 (KCNB1) expressed in HEK293 cells [11]. These differences could be due to differences in the cell type or expressed by other Kv channel mRNA. The native $I_{K}$ differ dramatically in terms of kinetics, amplitude, and drug response. Tissue and species–specific Kv gene expression contributes to the current heterogeneity [45]. HCFs also showed strong gene expression of Kv1.1, Kv1.2, and Kv3.1 in the Kv channels of these cells [51].

**Effect of CO on $I_{K}$ through variable signaling pathways**

**NOS/NO pathway:** To investigate the regulation of the $I_{K}$ channel by CO, the involvement of NO was first explored because CO and NO can cross-talk [54,55] and NO increases the expression of HO-1 [56,57].

We demonstrated that the activation of $I_{K}$ by CO in HCFs was abolished by pretreatment with NOS blockers (L-NMMA or L-NAME), which indicates that NO formation by CO–induced NOS stimulation has an important role in $I_{K}$ activation in HCFs by CO. These NO-dependent CO effects are also reported in the BK channels of HCFs [37] and L-type Ca$^{2+}$ channels of intestinal smooth muscle cells [22]. However, CO inhibits recombinant Kv1.5 expressed in HEK293 cells through NO [53].

NO also showed a similar difference for $I_{K}$. NO inhibited the hKv1.5 channel current, which activates $I_{K}$ in transfected Chinese hamster ovary cells and mouse ventricular myocytes [47] but also activated the current in HCFs [51], cardiomyocytes [58] and the sino-atrial node cells of guinea-pigs [59]. Considering the differing CO effects for $I_{K}$, the importance of HCFs in the heart cell population, and the connection between other cell types of the myocardium, more research is required on CO as a therapeutic agent for cardiovascular disease.

**sGC/cGMP/PKG pathway:** Previously, we demonstrated that CO-mediated augmentation of the BK channel is NO-dependent and involves channel S-nitrosylation and the PKG and PKA pathways [37].

The present study shows that CO activates the $I_{K}$ of HCFs through the cGMP-dependent pathway (Fig. 3A–C). These results are consistent with previous reports that CO binding can result in sGC activation, leading to the cellular generation of cGMP [60,61] and the CO effect for Kv1.5 expressed in HEK293 cells through sGC [53].

In case of NO, NO regulates diverse target proteins through different modes of post-transcriptional modification sGC/cGMP/PKG-dependent phosphorylation [62]. NO also blocks hKv1.5 channels by cyclic GMP-dependent mechanism [47].

**AC/cAMP/PKA pathway:** We have also shown that the cAMP-dependent pathway is involved in CO-mediated activation of $I_{K}$ in HCFs (Fig. 3D–F). Our results indicate that both the PKG and PKA pathways are involved in the CO-induced activation of $I_{K}$. NO also can regulate both adenylate cyclase and guanylate cyclase in cardiac myocytes and increase cAMP by activation of adenylate cyclase in cardiomyocytes [63]. CO also activates BK currents in HCFs through the cAMP-dependent pathway [37]. cAMP modulates significant alterations of cardiac electrical activity via a cGMP-dependent mechanism [64] and should be considered a novel regulator of cardiac electrophysiology.

**S-nitrosylation:** NO exerts ubiquitous signaling via post-translational modification of cysteine residues, a reaction termed S-nitrosylation. Specifically, S-nitrosylation modulates the major currents involved in the generation of the action potential and many cardiac ion channels such as the voltage-gated sodium channels, L-type Ca$^{2+}$ channels, and voltage-gated potassium channels [58].

NO regulates diverse target proteins through S-nitrosylation [62] and blocks hKv1.5 channels by S-nitrosylation [47]. CO also caused S-nitrosylation of Kv1.5 of HL-1 murine atrial cells [53], and elevated NO levels in myocytes, and S-nitrosylation of the Nav1.5 channel [65] and BK channel of HCFs [66]. However, S-nitrosylation is not involved in the NO-dependent activation of $I_{K}$ in HCFs [51]. Therefore, this pathway suggests that a direct reaction of CO or a consequence of some interaction of CO with other mechanisms (e.g., MAPK or the redox signaling pathways) is required to activate $I_{K}$ channels.

**MAPK pathway:** Although the underlying mechanism and specific molecular targets involved are unknown, there is a sig-
CO activation of delayed rectifier K+ currents through diverse pathways

significant body of evidence that indicates that CO can also interfere with MAPK-dependent pathway signaling [67,68]. MAPKs are involved in Kv channel modulation in VSMCs [69] and rat coronary arterial myocytes [38].

In our results, MAPK pathway inhibition with SB239063 (p38 MAPK inhibitor) or PD98059 (p44/42 MAPK inhibitor) depressed CO-induced $I_K$ stimulation. This suggests that MAPK pathway plays an important role in the CO-induced activation of $I_K$ channels in HCFs.

Activation of p38 MAPK by CO may involve upstream MAP kinase kinase-3 [12] or may involve the regulation of phosphatases or sGC activation (reviewed by [70]).

**Redox signaling pathways**: We also tested the redox signaling pathway for the CO effect because exposure to high amounts of CO inhibits mitochondrial respiration, generates ROS, and enhances ventricular arrhythmia after oxidative stress [71].

In our results, CO-induced $I_K$ activation was blocked by pretreatments with two superoxide dismutase mimetics: a nicotinamide adenine dinucleotide (NADPH) oxidase inhibitor, and a xanthine oxidase inhibitor (Fig. 5). CO-mediated activation of $I_K$ was partly attenuated by the SOD mimetics (MnTMPyP and MnTBP). These agents presumably increase $\text{H}_2\text{O}_2$ levels through superoxide dismutation, and $\text{H}_2\text{O}_2$ has an augmenting effect on the Kv1.5 channel [53].

CO increases ROS and enhances ventricular arrhythmia after oxidative stress [71]. In the heart, redox signaling regulates several physiological processes (e.g., excitation–contraction coupling) and is involved in a wide variety of pathophysiological and homeostatic or stress response pathways. ROS can be produced by a variety of enzyme systems associated with heart failure and is involved in cardiac redox signaling, derived from many sources, including xanthine oxidase [72], NOSs [73], and NADPH oxidases (NOXs) [74]. Among the ROS sources in the heart, NOXs are particularly important in redox signaling. NOX isoforms are expressed in multiple cell types, including cardiomyocytes and fibroblasts [75].

Modulation of the NOX1/NADPH oxidase signaling pathway may be a novel therapeutic strategy for preventing heart failure after myocardial injury [76]. NOX4-derived increase in ROS induces inhibition of the hKv1.5 channel [77]. HO-1 expression is increased in atrial fibrillation (AF) and appears to provide protection against the oxidative stress associated with this condition [78,79]. Given the important role of Kv1.5 in normal atrial function, its redox sensitivity and the likely involvement of HO-1 are protective in AF.

Data continues to establish CO as an important gasotransmitter alongside NO, which provides a range of beneficial cardiovascular effects. CO dilates coronary and other vessels [80] and HO-1 induction, which produces CO, protects against myocardial infarction, hypertension and vascular injury [68]. CO accounts for many of the effects of HO-1 induction [81-83] and CO inhalation, as well as CORMs, are being developed for cardiovascular therapy [7].

While several studies have shown the cardioprotective effect of CO or CORMs, their application has not yet reached clinical practice and their responses depend on the cell types [84,85].

Moreover, these variable responses to treatment in different tissues and organs could be accompanied by unexpected side effects. Therefore, it is still early to consider CO as a therapeutic, since it has only been shown to have a positive effect on specific individual organs. Our findings add to the growing understanding of the complexity of CO signaling in cardiac tissues by describing a new ion channel target for regulation.

In our experiments, CO significantly activated the $I_K$ of HCFs. Since the $I_K$ of HCFs was significantly inhibited by DPO-1, a specific Kv1.5 channel blocker, Kv1.5 channels appears to be the main component of $I_K$. Effects of CO on Kv1.5 was reported by Al-Owais et al. [53]. However, different from our results, the voltage-gated Kv1.5 channels of HEK293 and HL-1 cells are inhibited by CO [53]. Even though the effects of CO in experimental studies differ depending on the cell or tissue in question, further evaluation is required. We also found multiple redundant inhibitory effects of various pharmacological inhibitors on the CO effects on the $I_K$ of HCFs. It was very difficult to suggest a single line of signaling cascade to explain the results obtained by the inhibitors of signaling pathways. Our results may demonstrate the possible involvement of multiple mechanisms for the effect of CO on the $I_K$ of HCFs. Further studies are necessary to describe how CO modulates the currents in HCFs.

**FUNDING**

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2020R1A2C1007918).

**CONFLICTS OF INTEREST**

The authors declare no conflicts of interest.

**REFERENCES**

1. Ernst A, Zibrak JD. Carbon monoxide poisoning. *N Engl J Med*. 1998;339:1603-1608.
2. Ewing JF, Raju VS, Maines MD. Induction of heart heme oxygenase-1 (HSP32) by hyperthermia: possible role in stress-mediated elevation of cyclic 3’5’-guanosine monophosphate. *J Pharmacol Exp Ther*. 1994;271:408-414.
3. Lakkisto P, Palojoki E, Bäcklund T, Saraste A, Tikkanen I, Voipio-Pulkki LM, Pulkki K. Expression of heme oxygenase-1 in response to myocardial infarction in rats. *J Mol Cell Cardiol*. 2002;34:1357-1365.
4. Shan H, Li T, Zhang L, Yang R, Li Y, Zhang M, Dong Y, Zhou Y, Xu C, Yang B, Liang H, Gao X, Shan H. Heme oxygenase-1 prevents heart against myocardial infarction by attenuating ischemic injury-induced cardiomyocytes senescence. *Elife*. 2019;39:59-68.

5. Ndiasang JF, Chibbar R, Lane N. Heme oxygenase suppresses markers of heart failure and ameliorates cardiomyopathy in L-NAME-induced hypertension. *Eur J Pharmacol*. 2014;734:23-34.

6. Cheng Y, Rong J. Therapeutic potential of heme oxygenase-1/carbon monoxide system against ischemia-reperfusion injury. *Carr Pharm Des*. 2017;23:3884-3898.

7. Motterlini R, Otterbein LE. The therapeutic potential of carbon monoxide. *Nat Rev Drug Discov*. 2010;9:728-743.

8. Lamon BD, Zhang FF, Rong J. Therapeutic potential of heme oxygenase-1/carbon monoxide system against ischemia-reperfusion injury. *Carr Pharm Des*. 2017;23:3884-3898.

9. Motterlini R. Carbon monoxide-releasing molecules (CO-RMs): vasodilatory, anti-ischaemic and anti-inflammatory activities. *Biochem Soc Trans*. 2007;35(Pt 5):1142-1146.

10. Akamatsu Y, Haga M, Tyagi S, Yamashita K, Graça-Souza AV, Ollinger R, Csizmadia E, May GA, Ildigbo E, Otterbein LE, Bach FH, Soares MP. Heme oxygenase-1-derived carbon monoxide protects hearts from transplant associated ischemia reperfusion injury. *FASEB J*. 2004;18:771-772.

11. Dallas ML, Boyle JP, Milligan CJ, Sayer R, Kerrigan TL, McKinstry RJ, Flavell RA, Choi AM. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat Med*. 2006;6:422-428.

12. Li L, Hsu A, Moore PK. Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulphide in the cardiovascular system and in inflammation—a tale of three gases! *Pharmacol Ther*. 2009;123:386-400.

13. Grandi E, Sanguinetti MC, Bartos DC, Bers DM, Chen-Izu Y, Chiamvimonvat N, Colecraft HM, Delisle BP, Heijmen J, Navedel MF, Noskov S, Proenza C, Vandenberg JJ, Yarov-Yarovoy V, Potassium channels in the heart: structure, function and regulation. *J Physiol*. 2017;595:2209-2228.

14. Chen L, Sampson KJ, Kass RS. Cardiac delayed rectifier potassium channels in health and disease. *Card Electrophysiol Clin*. 2016;8:307-322.

15. Wang R, Wang Z, Wu L. Carbon monoxide-induced vasorelaxation and the underlying mechanisms. *Br J Pharmacol*. 1997;121:927-934.

16. Dallas ML, Scragg JL, Peers C. Modulation of hTREK-1 by carbon monoxide. *Neuroreport*. 2008;19:345-348.

17. Li L, Hsu A, Moore PK. Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulphide in the cardiovascular system and in inflammation—a tale of three gases! *Pharmacol Ther*. 2009;123:386-400.

18. Grandi E, Sanguinetti MC, Bartos DC, Bers DM, Chen-Izu Y, Chiamvimonvat N, Colecraft HM, Delisle BP, Heijmen J, Navedel MF, Noskov S, Proenza C, Vandenberg JJ, Yarov-Yarovoy V. Potassium channels in the heart: structure, function and regulation. *J Physiol*. 2017;595:2209-2228.

19. Chen L, Sampson KJ, Kass RS. Cardiac delayed rectifier potassium channels in health and disease. *Card Electrophysiol Clin*. 2016;8:307-322.

20. Wang R, Wang Z, Wu L. Carbon monoxide-induced vasorelaxation and the underlying mechanisms. *Br J Pharmacol*. 1997;121:927-934.

21. Dallas ML, Scragg JL, Peers C. Inhibition of L-type Ca²⁺ channels by carbon monoxide. *Adv Exp Med Biol*. 2009;648:89-95.

22. Lim I, Gibbons SJ, Lyford GL, Miller SM, Streege PR, Sarr MG, Chatterjee S, Szurszewski JH, Shah VH, Farrugia G. Carbon monoxide activates human intestinal smooth muscle L-type Ca²⁺ channels through a nitric oxide-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol*. 2005;288:G7-G14.

23. Scragg JL, Dallas ML, Wilkinson JA, Varadi G, Peers C. Carbon monoxide inhibits L-type Ca²⁺ channels via redox modulation of key cysteine residues by mitochondrial reactive oxygen species. *J Biol Chem*. 2008;283:24412-24419.

24. Camelli P, Borg TK, Kohl P. Structural and functional characterisation of cardiac fi broblasts. *Cardiovasc Res*. 2005;65:40-51.

25. Krenning G, Zeisberg EM, Kalluri R. The origin of fi broblasts and mechanism of cardiac fi brosis. *J Cell Physiol*. 2010;225:631-637.

26. Vliegen HW, van der Laarse A, Cornelisse CJ, Eulderink F. Myocardial changes in pressure overload-induced left ventricular hypertropy. A study on tissue composition, polyploidization and multinucleation. *Eur Heart J*. 1991;12:488-494.

27. Villarrreal FJ, Kim NN. Regulation of myocardial extracellular matrix components by mechanical and chemical growth factors. *Cardiovasc Pathol*. 1998;7:145-151.

28. Camelli P, Green CR, LeGrice I, Kohl P. Fibroblast network in rabbit sinoatrial node: structural and functional identification of homogeneous and heterogeneous cell coupling. *Circ Res*. 2004;94:828-835.

29. Kohl P. Heterogeneous cell coupling in the heart: an electrophysiological role for fi broblasts. *Circ Res*. 2003;93:381-383.

30. Gaudesius G, Miragoli M, Thomas SP, Rohr S. Coupling of cardiac electrical activity over extended distances by fi broblasts of cardiac origin. *Circ Res*. 2003;93:421-428.

31. Asada K, Kurokawa J, Furukawa T. Redox- and calmodulin-dependent S-nitrosylation of the KCNQ1 channel. *J Biol Chem*. 2009;284:6014-6020.

32. Li GR, Sun HY, Chen JB, Zhou Y, Tse HF, Lau CP. Characterization of multiple ion channels in cultured human cardiac fi broblasts. *PloS One*. 2009;4:e7307.

33. Yue L, Xie J, Nattel S. Molecular determinants of cardiac fi broblast electrical function and therapeutic implications for atrial fi brillation. *Cardiovasc Res*. 2011;89:744-753.

34. Miragoli M, Salvareni N, Rohr S. Myofibroblasts induce ectopic activity in cardiac tissue. *Circ Res*. 2007;101:755-758.

35. Pellman J, Zhang J, Sheikh F. Myocyte-fi broblast communication in cardiac fi brosis and arrhythmias: mechanisms and model systems. *J Mol Cell Cardiol*. 2016;94:22-31.

36. Bae H, Lee D, Kim YW, Choi J, Lee HJ, Kim SW, Noh YH. MAPKs-mediated modulation of the myocyte voltage-gated K⁺ channels is involved in ethanol-induced rat coronary arterial contraction. *Eur J Pharmacol*. 2018;834:274-280.
39. Lagrutta A, Wang J, Fermini B, Salata JJ. Novel, potent inhibitors of human Kv1.5 K⁺ channels and ultrarapidly activating delayed rectifier potassium current. J Pharmacol Exp Ther. 2006;317:1054-1063.

40. Wang Z, Fermini B, Nattel S. Delayed rectifier outward current and repolarization in human atrial myocytes. Circ Res. 1993;73:276-285.

41. Wang Z, Fermini B, Nattel S. Sustained depolarization-induced outward current in human atrial myocytes. Evidence for a novel delayed rectifier K⁺ current similar to Kv1.5 cloned channel currents. Circ Res. 1993;73:1061-1076.

42. Abderrahmane A, Salvail D, Dumoulin M, Garon J, Cadieux A, Rousseau E. Direct activation of Kᵦ channel in airway smooth muscle by nitric oxide: involvement of a nitrothiolsylation mechanism? Am J Respir Cell Mol Biol. 1998;19:485-497.

43. Heijman J, Algalarrondo V, Voigt N, Melka J, Wehrens XH, Dobrev D, Nattel S. The value of basic research insights into atrial fibrillation mechanisms as a guide to therapeutic innovation: a critical analysis. Cardiovasc Res. 2016;109:467-479.

44. Ravens U, Wettwer E. Ultra-rapid delayed rectifier channels: molecular basis and therapeutic implications. Cardiovasc Res. 2011;89:776-785.

45. Ko EA, Park WS, Firth AL, Kim N, Yuan JX, Han J. Pathophysiology of voltage-gated K⁺ channels in vascular smooth muscle cells: modulation by protein kinases. Prog Biophys Mol Biol. 2010;103:95-101.

46. Feng J, Wible B, Li GR, Wang Z, Nattel S. Antisense oligodeoxynucleotides directed against Kv1.5 mRNA specifically inhibit ultrarapid delayed rectifier K⁺ current in cultured adult human atrial myocytes. Circ Res. 1997;80:572-579.

47. Núñez L, Vaquero M, Gómez R, Caballero R, Mateos-Cáceres P, Macaya C, Iriepa I, Gálvez E, López-Farré A, Tamargo J, Delpón E. Nitric oxide blocks hKv1.5 channels by S-nitrosylation and by a cyclic GMP-dependent mechanism. Cardiovasc Res. 2006;72:80-89.

48. Schmitt N, Grunnet M, Olesen SP. Cardiac potassium channel subtypes: new roles in repolarization and arrhythmia. Physiol Rev. 2014;94:609-653.

49. Wettwer E, Terlau H. Pharmacology of voltage-gated potassium channel Kv1.5--impact on cardiac excitability. Curr Opin Pharmacol. 2014;15:115-121.

50. Ravens U, Odening KE. Atrial fibrillation: therapeutic potential of atrial K⁺ channel blockers. Pharmacol Ther. 2017;176:13-21.

51. Bae H, Choi J, Kim YW, Lee D, Kim JH, Ko JH, Bang H, Kim T, Lim K. Effects of nitric oxide on voltage-gated K⁺ currents in human cardiac fibroblasts through the protein kinase G and protein kinase A pathways but not through S-nitrosylation. Int J Mol Sci. 2018;19:814.

52. Ordög B, Brutýo E, Puskás LG, Papp JG, Varró A, Szabó J, Boldogh Z. Gene expression profiling of human cardiac potassium and sodium channels. Int J Cardiol. 2006;111:386-393.

53. Al-Owais MM, Hettiarachchi NT, Boyle JP, Scragg JL, Elies J, Dallas ML, Lippiat JD, Steele DS, Peers C. Multiple mechanisms mediating carbon monoxide inhibition of the voltage-gated K⁺ channel Kv1.5. Cell Death Dis. 2017;8:e3163.

54. Hartsfield CL. Cross talk between carbon monoxide and nitric oxide. Antioxid Redox Signal. 2002;4:301-307.

55. Ingi T, Cheng J, Ronnett GV. Carbon monoxide: an endogenous modulator of the nitric oxide-cyclic GMP signaling system. Neuron. 1996;16:835-842.

56. Durante W, Christodoulides N, Cheng K, Peyton KJ, Sunahara RK, Schafer AI. cAMP induces heme oxygenase-1 gene expression and carbon monoxide production in vascular smooth muscle. Am J Physiol. 1997;273(1 Pt 2):H317-H323.

57. Polte T, Abate A, Denner PY, Schröder H. Heme oxygenase-1 is a cGMP-inducible endothelial protein and mediates the cytoprotective action of nitric oxide. Arterioscler Thromb Vasc Biol. 2000;20:1209-1215.

58. Bai CX, Takahashi K, Masumiya H, Sawanobori T, Furukawa T. Nitric oxide-dependent modulation of the delayed rectifier K⁺ current and the L-type Ca⁺² current by ginsenoside Re, an ingredient of Panax ginseng, in guinea-pig cardiomyocytes. Br J Pharmacol. 2004;142:567-575.

59. Shimizu K, Shintani Y, Ding WG, Matsuura H, Bamba T. Potentiation of slow component of delayed rectifier K⁺ current by cGMP via two distinct mechanisms: inhibition of phosphodiesterase 3 and activation of protein kinase G. Br J Pharmacol. 2002;137:127-137.

60. Brüne B, Ullrich V. Inhibition of platelet aggregation by carbon monoxide is mediated by activation of guanylate cyclase. Mol Pharmacol. 1987;32:497-504.

61. Kharitonov VG, Sharma VS, Pilz RB, Magde D, Koelsing SL. Basis of guanylate cyclase activation by carbon monoxide. Proc Natl Acad Sci U S A. 1995;92:2568-2571.

62. Zhang YH. Nitric oxide signalling and neuronal nitric oxide synthase in the heart under stress. Philos Trans. 2017;6:742.

63. Vila-Petroff MG, Yones A, Egan J, Lakatta EG, Sollott SJ. Activation of distinct cAMP-dependent and cGMP-dependent pathways by nitric oxide in cardiac myocytes. Circ Res. 1999;84:1020-1031.

64. Abramochkin DV, Konovalova OP, Kamkin A, Sittidikova GF. Carbon monoxide modulates electrical activity of murine myocardium via cGMP-dependent mechanisms. J Physiol Biochem. 2015;71:107-119.

65. Dallas ML, Yang Z, Boyle JP, Boycott HE, Scragg JL, Milligan CJ, Elies J, Duke A, Thireau J, Rebuffel C, Richard S, Bernus O, Steele DS, Peers C. Carbon monoxide induces cardiac arrhythmia via induction of the late Na⁺ current. Am J Respir Crit Care Med. 2012;186:648-656.

66. Bae H, Lim I. Effects of nitric oxide on long-conductance Ca²⁺-activated K⁺ currents in human cardiac fibroblasts through PKA and PKG-related pathways. Clin Exp Pharmacol Physiol. 2017;44:1116-1124.

67. Kim HP, Ryter SW, Choi AM. CO as a cellular signaling molecule. Annu Rev Pharmacol Toxicol. 2006;46:411-449.

68. Ryter SW, Alam J, Choi AM. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiol Rev. 2006;86:383-650.

69. Ishii T, Warabi E, Siow RCM, Mann GE. Sequestosome1/p62: a regulator of redox-sensitive voltage-activated potassium channels, arterial remodeling, inflammation, and neurite outgrowth. Free Radic Biol Med. 2013;65:102-116.

70. Boczkowski J, Poderoso JJ, Motterlini R. CO-metal interaction: vital role in the biology and pathology of Panax ginseng, in guinea-pig cardiomyocytes. Br J Pharmacol. 2004;142:567-575.

71. Ordög B, Brutýo E, Puskás LG, Papp JG, Varró A, Szabó J, Boldogh Z. Gene expression profiling of human cardiac potassium and sodium channels. Int J Cardiol. 2006;111:386-393.

72. Al-Owais MM, Hettiarachchi NT, Boyle JP, Scragg JL, Elies J, Dallas ML, Lippiat JD, Steele DS, Peers C. Multiple mechanisms mediating carbon monoxide inhibition of the voltage-gated K⁺ channel Kv1.5. Cell Death Dis. 2017;8:e3163.

73. Hartsfield CL. Cross talk between carbon monoxide and nitric oxide. Antioxid Redox Signal. 2002;4:301-307.

74. Ingi T, Cheng J, Ronnett GV. Carbon monoxide: an endogenous modulator of the nitric oxide-cyclic GMP signaling system. Neuron. 1996;16:835-842.

75. Durante W, Christodoulides N, Cheng K, Peyton KJ, Sunahara RK, Schafer AI. cAMP induces heme oxygenase-1 gene expression and carbon monoxide production in vascular smooth muscle. Am J Physiol. 1997;273(1 Pt 2):H317-H323.

76. Polte T, Abate A, Denner PY, Schröder H. Heme oxygenase-1 is a cGMP-inducible endothelial protein and mediates the cytoprotective action of nitric oxide. Arterioscler Thromb Vasc Biol. 2000;20:1209-1215.

77. Bai CX, Takahashi K, Masumiya H, Sawanobori T, Furukawa T. Nitric oxide-dependent modulation of the delayed rectifier K⁺ current and the L-type Ca²⁺ current by ginsenoside Re, an ingredient of Panax ginseng, in guinea-pig cardiomyocytes. Br J Pharmacol. 2004;142:567-575.
72. Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO, Kobeissi ZA, Marbán E, Hare JM. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. Circulation. 2001;104:2407-2411.
73. Takimoto E, Champion HC, Li M, Ren S, Rodriguez ER, Tavazzi B, Lazzarino G, Paolocci N, Gabrielson KL, Wang Y, Kass DA. Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load. J Clin Invest. 2005;115:1221-1231.
74. Heymes C, Bendall JK, Ratajczak P, Cave AC, Samuel JL, Hasenfuss G, Shah AM. Increased myocardial NADPH oxidase activity in human heart failure. J Am Coll Cardiol. 2003;41:2164-2171.
75. Nabebaccus A, Zhang M, Shah AM. NADPH oxidases and cardiac remodelling. Heart Fail Rev. 2011;16:5-12.
76. Iwata K, Matsuno K, Murata A, Zhu K, Fukui H, Ikuta K, Katsuyama M, Ibi M, Matsumoto M, Ohigashi M, Wen X, Zhang J, Cui W, Yabe-Nishimura C. Up-regulation of NOX1/NADPH oxidase following drug-induced myocardial injury promotes cardiac dysfunction and fibrosis. Free Radic Biol Med. 2018;120:277-288.
77. Mittal M, Gu XQ, Pak O, Pamerter ME, Haag D, Fuchs DB, Schermuly RT, Ghofrani HA, Brandes RP, Seeger W, Grimminger F, Haddad GG, Weissmann N. Hypoxia induces Kv channel current inhibition by increased NADPH oxidase-derived reactive oxygen species. Free Radic Biol Med. 2012;52:1033-1042.
78. Corradi D, Callegari S, Maestri R, Benussi S, Bosio S, De Palma G, Alinovi R, Cagliari A, Goldoni M, Mozzi P, Pastori P, Manotti L, Nascimbene S, Dorigo E, Rusconi R, Astorri E, Alfieri O. Heme oxygenase-1 expression in the left atrial myocardium of patients with chronic atrial fibrillation related to mitral valve disease: its regional relationship with structural remodeling. Hum Pathol. 2008;39:1162-1171.
79. Yeh YH, Hsu LA, Chen YH, Kuo CT, Chang GJ, Chen WJ. Protective role of heme oxygenase-1 in atrial remodeling. Basic Res Cardiol. 2016;111:58.
80. Leffler CW, Parfenova H, Jaggar JH, Wang R. Carbon monoxide and hydrogen sulfide: gaseous messengers in cerebrovascular circulation. J Appl Physiol (1985). 2006;100:1065-1076.
81. Durante W, Johnson FK, Johnson RA. Role of carbon monoxide in cardiovascular function. J Cell Mol Med. 2006;10:672-686.
82. Durante W, Kroll MH, Christodoulides N, Peyton KJ, Schafer AI. Nitric oxide induces heme oxygenase-1 gene expression and carbon monoxide production in vascular smooth muscle cells. Circ Res. 1997;80:557-564.
83. Otterbein LE, Foresti R, Motterlini R. Heme oxygenase-1 and carbon monoxide in the heart: the balancing act between danger signaling and pro-survival. Circ Res. 2016;118:1940-1959.
84. Johnson FK, Johnson RA. Carbon monoxide promotes endothelium-dependent constriction of isolated gracilis muscle arterioles. Am J Physiol Regul Integr Comp Physiol. 2003;285:R536-R541.
85. Lee TS, Chau LY. Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. Nat Med. 2002;8:240-246.