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Heme oxygenase-1 (HO-1)/carbon monoxide (CO) axis suppresses RANKL-induced osteoclastic differentiation by inhibiting redox-sensitive NF-κB activation

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Heme oxygenase (HO-1) catalyzes heme to carbon monoxide (CO), biliverdin/bilirubin, and iron and is known to prevent the pathogenesis of several human diseases. We assessed the beneficial effect of heme degradation products on osteoclastogenesis induced by receptor activator of NF-κB ligand (RANKL). Treatment of RAW264.7 cells with CORM-2 (a CO donor) and bilirubin, but not with iron, decreased RANKL-induced osteoclastogenesis, with CORM-2 having a more potent anti-osteogenic effect. CORM-2 also inhibited RANKL-induced osteoclastogenesis and osteoclastic resorption activity in marrow-derived macrophages. Treatment with hemin, a HO-1 inducer, strongly inhibited RANKL-induced osteoclastogenesis in wild-type macrophages, but was ineffective in HO-1$^{+/-}$ cells. CORM-2 reduced RANKL-induced NFATc1 expression by inhibiting IKK-dependent NF-κB activation and reactive oxygen species production. These results suggest that CO potently inhibits RANKL-induced osteoclastogenesis by inhibiting redox-sensitive NF-κB-mediated NFATc1 expression. Our findings indicate that HO-1/CO can act as an anti-resorption agent and reduce bone loss by blocking osteoclast differentiation. [BMB Reports 2017; 50(2): 103-108]

INTRODUCTION

Bone homeostasis is a strictly regulated physiological process that involves the formation and resorption of bone by osteoblast and osteoclast. Enhanced osteoclast formation (osteoclastogenesis) is an underlying mechanism in osteolytic bone diseases, including osteoporosis, rheumatoid arthritis, and bone metastasis. Osteoclasts are specialized multinucleated giant cells derived from mononuclear progenitors of the monocytes/macrophages lineage through a differentiation process that is mainly modulated by receptor activator of nuclear factor-κB (RANKL) and macrophage colony-stimulating factor (M-CSF) (1, 2).

RANKL-induced osteoclast differentiation is modulated by TNF receptor-associated factor 6 (TRAF 6)-mediated multiple pathways, including stimulation of the transcription factors nuclear factor-κB (NF-κB) through IκB kinase (IKK) activation and the activator protein-1 (AP-1) complex containing c-Fos through mitogen-activated protein kinase (MAPK) (3, 4). These transcription factors activate the transcription of the transcription factor nuclear factor of activated T cells-c1 (NFATc1), which upregulates the osteoclast-specific gene expression, such as cathepsin K (CtsK), tartrate-resistant acid phosphatase (TRAP), matrix metalloproteinase-9 (MMP-9), and calcitonin receptor (5). Hence, inhibition of the RANKL-induced signal pathway or osteoclast differentiation might be a potential strategy to treat bone diseases associated with excessive osteoclastogenesis.

Heme oxygenase (HO) is a rate-limiting enzyme of heme degradation, resulting in the liberation of iron, biliverdin/bilirubin, and carbon monoxide (CO). Among them, CO has pleiotropic biological functions, such as vasorelaxation, neurotransmission, antioxidation, anti-inflammation, and anti-apoptosis (6). Other heme catabolites, such as biliverdin, bilirubin, and iron, also have several biological activities similar to those exerted by CO (6, 7). Although some studies have proposed that CO regulates RANKL-induced osteoclastogenesis (8-10), its underlying mechanism is not clearly elucidated. In addition, the regulatory effects of other heme catabolites on osteoblast differentiation have also not been studied.

In the present study, we examined the effects of heme degradation products on RANKL-induced osteoclast differentiation and its underlying mechanism in RAW264.7 cells and

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mouse marrow-derived monocytes/macrophages cells (BMMs). Our results indicate that CO and bilirubin, but not iron, inhibits RANKL-induced osteoclastogenesis, with CO having a greater potent anti-osteogenic effect than bilirubin, by inhibiting redox-sensitive NF-κB-dependent NFATc1 expression. These results suggest that the HO-1/CO pathway has a potential role in preventing bone diseases associated with excessive osteoclast activity.

RESULTS

Effects of CO, iron, and bilirubin on RANKL-induced osteoclastogenesis

To evaluate the effects of heme degradation products on osteoblast differentiation, we examined the effects of CO, iron (Fe^{2+}), and bilirubin on RANKL-induced osteoclastogenesis. Briefly, murine macrophage RAW264.7 cells were pretreated with or without 50 μM CORM-2 (a CO-releasing compound), iron or bilirubin for 1 h, followed by co-stimulation with RANKL (100 ng/ml) for 5 days. CORM-2 effectively inhibited RANKL-induced increase in TRAP-positive multinucleated cells, a typical marker of osteoclastogenesis (Fig. 1A and B). Bilirubin partially suppressed osteoclast differentiation compared with the control RANKL alone. However, iron failed to significantly affect the RANKL-induced osteoclastogenic effects (Fig. 1A and B). The inhibitory effect of CORM-2 was dose-dependent, whereas the control compound $RuCl_3$, was ineffective on RANKL-induced osteoclastogenesis (Fig. 1C and D). These results suggest that CO potentially inhibits RANKL-induced osteoclastogenesis in immortalized macrophage RAW264.7 cells. Next, we examined the effect of CORM-2 on osteoclastogenesis in primary cultured mouse macrophages. Treatment with CORM-2 effectively abrogated the RANKL-induced increase in osteoclastogenesis of BMMs in a concentration-dependent manner (Fig. 1E and F). We further investigated whether CO regulates the bone resorption ability of BMMs stimulated with RANKL. When stimulated with RANKL on calcium phosphate-coated plates, BMMs increased the number of pits as a result of calcium resorption by functionally active osteoclasts differentiated from RANKL-treated BMMs; this pit formation was inhibited by CORM-2 in a concentration-dependent manner (Fig. 1G and H). These results suggest that CO inhibits RANKL-induced osteoclast differentiation and bone resorption.

CO suppresses RANKL-induced osteoclast marker gene expression

RANKL induces osteoclast differentiation by increasing the expression levels of NFATc1 and the AP-1 component c-Fos, which stimulate the expression of osteoblast-specific genes (4). Western blot and qRT-PCR analyses showed that RANKL significantly increased the expression level of NFATc1, but not c-Fos. This increase was abrogated by treatment with CORM-2 (Fig. 2A and B). Furthermore, CORM-2 treatment significantly

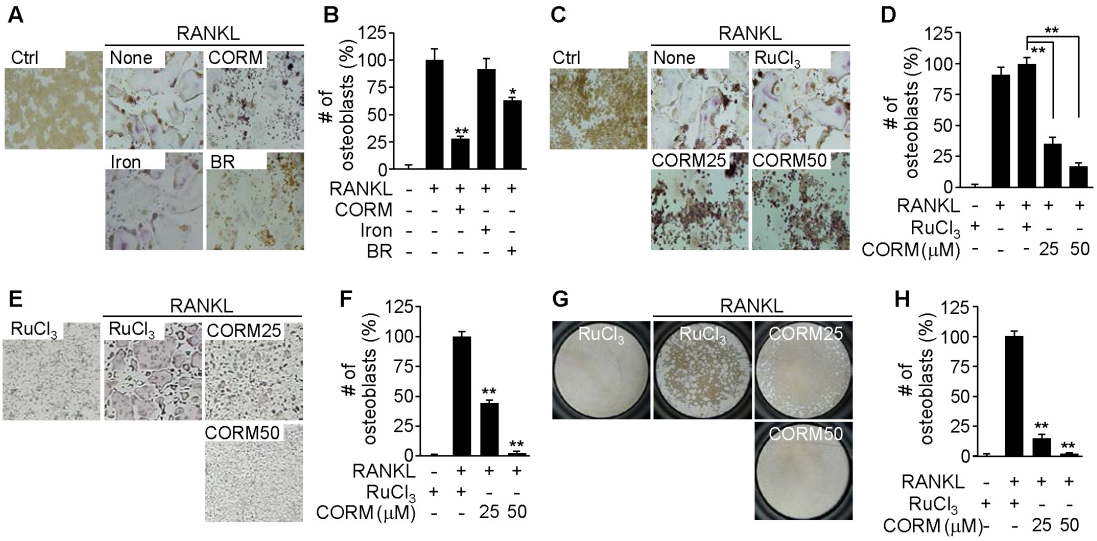

Fig. 1. Effects of CO, iron, and bilirubin on RANKL-induced osteoclastogenesis. (A-D) RAW264.7 cells were pretreated for 1 h with the indicated concentrations or 50 μM of CORM-2 (CORM), ferrous iron, bilirubin (BR) or 100 μM $RuCl_3$, followed by co-stimulation with RANKL (100 ng/ml) for 5 days. (A and C) Cells were stained with TRAP reagent. (B and D) TRAP-positive multinucleated cells were counted using an optical microscope. (E-H) Mouse BMMs cultured on normal (E and F) or calcium- phosphate-coated plate (G and H) were treated with M-CSF and RANKL in the presence or absence of 100 μM $RuCl_3$ or 50 μM CORM-2 for 5 days. (E) Cells were stained with TRAP reagent. (F) TRAP-positive cells were counted. (G and H) Resorption pit areas were determined after 7 days and quantitated using image analyzing software (Image proplus™). *$P < 0.05$ and **$P < 0.01$ versus RANKL alone or in combination with $RuCl_3$.

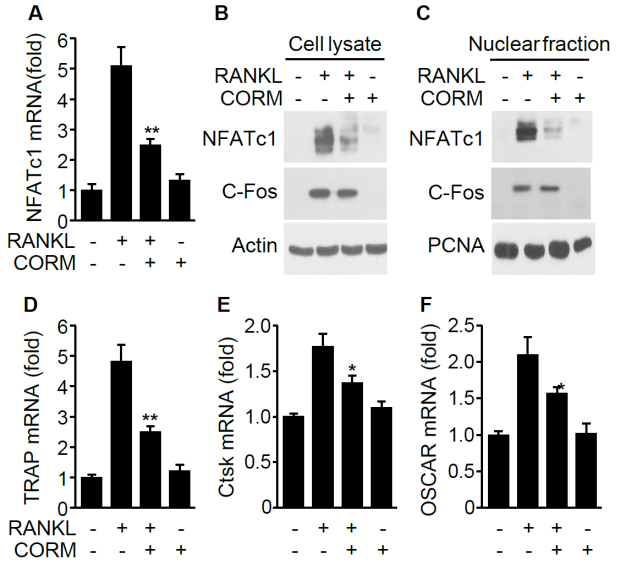

Fig. 2. CO inhibits RANKL-induced NFATc1 and osteoclast marker gene expression. RAW264.7 cells were pretreated with CORM-2 (CORM, 50 μM) for 1 h and stimulated with RANKL (100 ng/ml). (A) After 24 h, NFATc1 and β-actin mRNA levels were determined by RT-PCR analysis and quantitated by densitometry. (B and C) After 24 h, NFATc1 and C-Fos levels in whole cell extracts and nuclear fractions were determined by Western blotting. (D-F) After 72 h, mRNA levels of TRAP, CtsK, and OSCAR were determined by RT-PCR analysis and quantitated by densitometry. *P < 0.05 and **P < 0.01 *versus* RANKL alone.

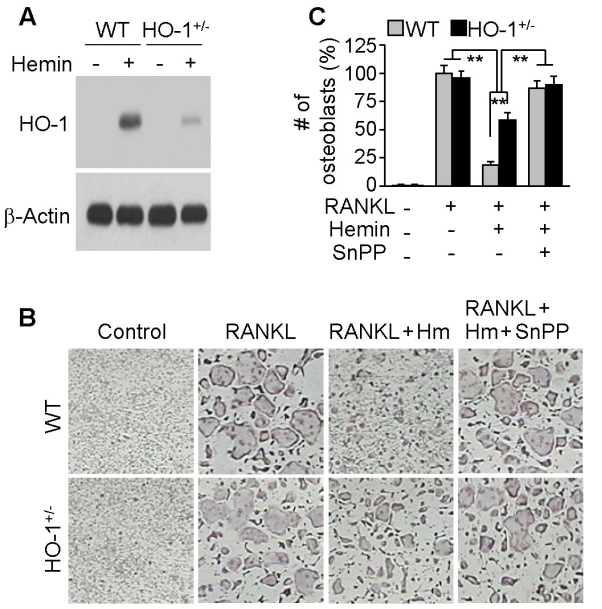

Fig. 3. Hemin-induced HO-1 inhibits RANKL-mediated osteoclastogenesis. (A-C) BMMs isolated wild-type (WT) and HO-1$^{+/-}$ mice were treated with hemin (2 μM) for 8 h (A), followed by stimulation with M-CSF and RANKL in the presence or absence of SnPP (1 μM) for 5 days. (A) HO-1 levels were determined by Western blotting. (B and C) TRAP-positive multinucleated cells were counted through optical microscopy.

decreased the protein levels of NFATc1 in the nuclear fraction, but not c-Fos (Fig. 2C). Under the same experimental conditions, NFATc1-dependent osteoblast marker genes, such as TRAP, Ctsk, and OSCAR, were induced in response to RANKL, and these increases were significantly blocked by CORM-2 (Fig. 2D-F). These data indicate that CO suppresses the osteoblast-specific marker gene expression by inhibiting RANKL-induced expression of NFATc1.

Heme-mediated HO-1 induction suppresses RANKL-induced osteoclastogenesis

To examine whether endogenous CO regulates RANKL-induced osteoclastogenesis, BMMs were treated with hemin (a substrate and potent inducer of HO-1) to elicit HO-1 induction, followed by stimulation with RANKL in the presence of M-CSF for 5 days. When treated with hemin, HO-1 was strongly induced in BMMs from wild-type mice, but weakly expressed in cells from HO-1$^{+/-}$ mice (Fig. 3A). Hemin treatment strongly inhibited RANKL-induced osteoclastogenesis in BMMs from wild-type, but was not effective in cells from HO-1$^{+/-}$ mice; these inhibitions were reversed by the HO-1 inhibitor SnPP (Fig. 3B and C). These results suggest that HO-1-derived CO plays an important role in negative regulation of osteoclastogenesis.

CO inhibits RANKL-induced activation of NF-κB, but not MAPKs or Akt

During osteoclast differentiation, NFATc1 expression is dependent on transcription factors, such as AP-1 and NF-κB, which get activated by stimulating MAPKs and IκB degradation, respectively (4, 5). We assessed the effects of CO on RANKL-induced activation of MAPKs. RANKL increased phosphorylation-dependent activation of ERK, JNK, and p38, and these activations were not significantly affected by CORM-2 treatment (Fig. 4A). Next, we evaluated the effects of CO on RANKL-induced NF-κB activation via IκB degradation elicited by activating IKK, NIK, and Akt (11). RANKL increased phosphorylation of IKKα/β (Ser180/181) and Akt, but not NIK (Fig. 4B). CORM-2 treatment inhibited RANKL-induced phosphorylation of IKKα/β, but not Akt, resulting in the suppression of RANKL-mediated IκBα (Ser32) phosphorylation and degradation (Fig. 4B). As a result, CORM-2 blocked RANKL-induced nuclear translocation of the NF-κB p65 subunit (Fig. 4C and D). Inhibition of the NF-κB pathway by CORM-2 was highly correlated with its suppressive effect on RANKL-induced reactive oxygen species (ROS) production (Fig. 4E), suggesting that CO inhibits RANKL-induced redox-sensitive NF-κB activation. Moreover, CORM-2 abrogated RANKL-induced NF-κB reporter activity (Fig. 4F). We further examined whether CORM-2 could regulate NF-κB binding to the κB element at

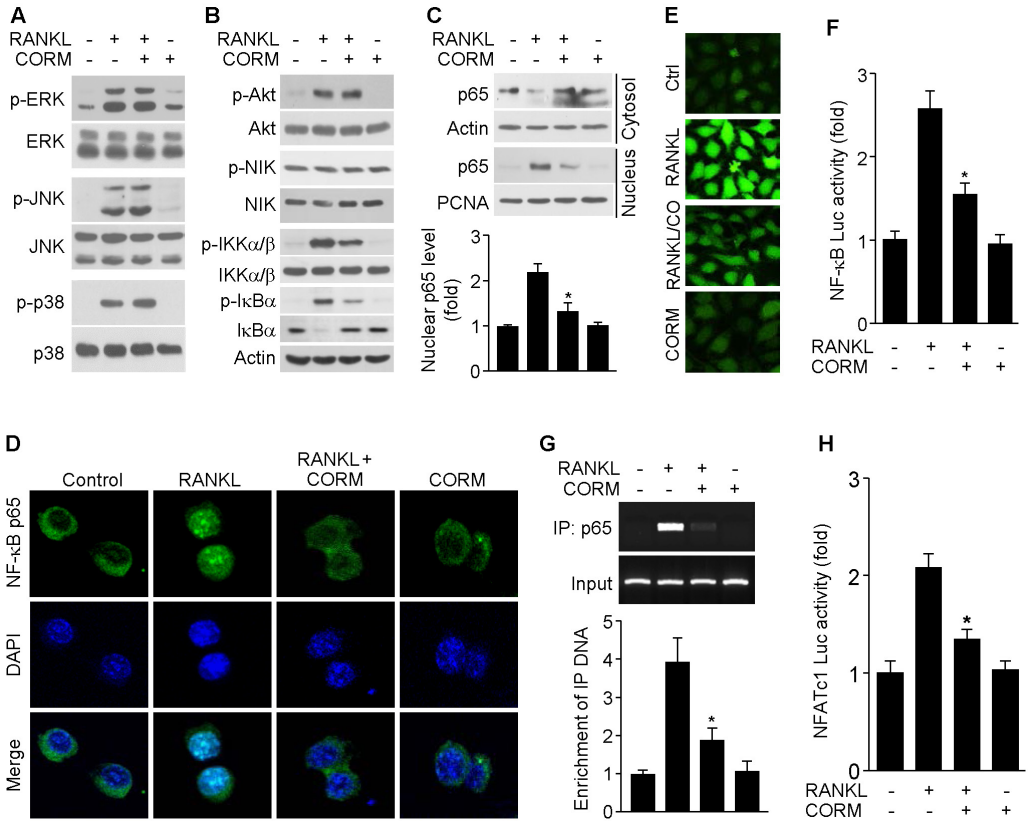

Fig. 4. CO inhibits RANKL-induced activation of NF-κB, but not MAPKs or Akt. RAW264.7 cells were treated with RANKL (100 ng/ml) alone or in combination with CORM-2 (CORM, 50 μM). (A and B) After 30 min, phosphorylated levels of MAPKs (A) and NF-κB signal mediators (B) were determined by Western blotting. (C) After 1 h, NF-κB p65 levels were determined in the cytosolic and nuclear fractions by Western blotting. (D) Nuclear translocation of p65 was determined by confocal laser microscopy. (E) After 20 min, intracellular ROS levels were determined by confocal microscopy using DCF-DA. (F) Cells were transfected with a NF-κB-Luc construct, followed by treatment with RANKL alone or in combination with CORM-2 for 24 h. Luciferase activity was determined in cell lysates. (G) NF-κB binding to NFATc1 promoter was determined by ChIP analysis. (H) NFATc1 promoter activity was determined by luciferase-reporter gene expression assay. *P $<$ 0.05 *versus* RANKL alone.

-664 bp upstream in the murine NFATc1 promoter (5). Chromatin immunoprecipitation (ChIP) analysis showed that CORM-2 significantly decreased RANKL-induced binding of NF-κB to NFATc1 promoter (Fig. 4G). In addition, CORM-2 inhibited RANKL-induced transcriptional activity of NFATc1 promoter (Fig. 4H). These results indicate that CO inhibits the RANKL-induced NF-κB signal pathway by inhibiting IKK activation, resulting in the inhibition of NFATc1 expression.

DISCUSSION

This study was undertaken to elucidate the potential inhibitory effect and molecular mechanism of heme catabolites on RANKL-induced osteoclastic differentiation, a key process in bone metabolism. We found that exogenous CO (generated by CORM-2) and bilirubin, but not iron, suppressed RANKL-induced osteoclastogenesis or osteoclastic bone resorption activity in RAW264.7 cells and BMMs, with CO being potently more effective than bilirubin. Similar anti-osteoclastogenic effect was observed in wild-type BMMs treated with hemin, a HO-1 inducer; however, the effect of hemin was much less in HO-$1^{+/-}$ cells. We also observed that the anti-osteoclastogenic activity of CO was associated with inhibition of redox-sensitive NF-κB-dependent NFATc1 expression, leading to the suppression of NFATc1-mediated expression of osteoclast-specific genes. These results suggest that the HO-1/CO axis potentially restrains osteoclastogenesis and prevents bone loss by inhibiting NF-κB-dependent NFATc1 expression.

HO-1 is recognized as a cytoprotective, antioxidant, and anti-inflammatory gene by catabolizing heme to biliverdin (finally converts to bilirubin), iron, and CO (7). Among these products, CO plays the most important role in HO-1-mediated

biological functions (12). Other metabolites have similar biological activity through ROS scavenging or induction of the antioxidant gene ferritin (7, 12). Previous studies have demonstrated that exogenous CO gas or CO-releasing molecules have a beneficial effect as therapeutic treatment in animal models of inflammatory diseases and traumatic brain injury (13, 14). Moreover, a number of studies have also shown that HO-1 expression or CO delivery prevents inflammatory arthritis and bone loss in animal models (8-10), probably by suppressing either inflammatory cytokine production or osteoclast differentiation. Similarly, we found that induction of HO-1 or treatment with CORM-2 or bilirubin, but not iron, prevented RANKL-induced osteoclast differentiation, with CO having more potent anti-osteoclastogenic activity compared to bilirubin. This indicates that, among HO-1 metabolites, CO is a major contributor to the inhibitory effect of HO-1 on osteoblast differentiation.

RANKL induces osteoclast differentiation from BMMs by triggering the intracellular signal pathway that promotes induction or activation of transcription factors, such as NFATc1 and NF-κB (4, 5). NFATc1 is a key transcription factor in osteoclast differentiation. Thus, Nfatc1$^{-/-}$ embryonic stem cells are unable to differentiate into osteoclasts *in vitro* and overexpression of NFATc1 induces osteoclastogenesis (15). It is evident that NFATc1 is an inducible gene that acts as a master transcription factor for RANKL-induced osteoblast differentiation. In fact, NFATc1 binds to its own promoter and is robustly induced through an autoamplification circuit (5). NFATc1 promoter also cooperatively interacts with other RANKL-stimulated transcription factors, such as NF-κB and AP-1 to activate the initial induction of NFATc1, followed by an autoamplification phase of NFATc1. This indicates that activation of NF-κB and AP-1 plays an important role in RANKL-induced osteoclastogenesis (5). This study revealed that CO inhibits RANKL-induced NFATc1 expression by suppressing IKK-dependent NF-κB activation, but not the AP-1 component c-Fos expression, resulting in the suppression of NFATc1-targeted osteoclast marker genes, including TRAP, Ctsk, and OSCA. On the other hand, RANKL also activates MAP kinase (MAPK) and Akt to induce the expression of critical genes for osteoclast differentiation (4). In general, NF-κB is activated by several upstream signal mediators, such as IKK, Akt, and MAPKs (16). However, our data showed that, of the upstream signal mediators activated by RANKL, only IKK was inhibited by CO. More interestingly, CORM-2 inhibited RANKL-induced ROS generation upstream of IKK (16), suggesting that CO dampens ROS-dependent IKK activation. These results suggest that CO inhibits RANKL-mediated NFATc1 autoamplification and osteoclastogenesis through initial suppression of the ROS/IKK/NF-κB pathway, but not other signal mediators.

CO inhibits NF-κB activation in response to various inflammatory stimuli, resulting in the prevention of inflammatory diseases. Several mechanisms have been proposed to explain the inhibitory effect of CO-mediated NF-κB activation. First, CO inhibits the redox-sensitive NF-κB signaling pathway by decreasing ROS production via inhibition of NADPH oxidase activation in response to LPS or TNF-α (17, 18). Interestingly, RANKL increases NADPH oxidase-dependent ROS production, which stimulates osteoblast differentiation by activating NF-κB (19, 20). Consistent with previous evidence that CO and bilirubin have potent antioxidant activity (7, 21), we found that CORM-2 inhibited RANKL-induced ROS generation. Thus, it is possible that CO inhibits RANKL-mediated NADPH oxidase activation and ROS accumulation, resulting in inhibition of redox-sensitive IKK-mediated NF-κB activation. The anti-osteogenic effect shown by bilirubin might be associated with the inhibition of NF-κB activation via its antioxidant activity which inhibits ROS generation and IKK activation (19, 20), although these evidence have not been provided in this study. Another possibility is associated with S-glutathiolation of the NF-κB p65 subunit, which is independent of IKK activation (22). Our data showed that CO inhibited IKK-dependent IκBα phosphorylation and degradation, indicating that CO may be unable to stimulate the S-glutathiolation of p65 in macrophages stimulated with RANKL.

Taken together, our data demonstrate that CO inhibits RANKL-induced osteoclastogenesis by suppressing the NF-κB-dependent NFATc1 expression, probably by dampening ROS production. Our results provide new insight into the therapeutic potential of CO for treating abnormal bone metabolism and osteolytic bone diseases.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM), α-minimum essential medium (α-MEM), and supplements were purchased from Invitrogen Life Technologies (Carlsbad, CA). Fetal bovine serum (FBS) was obtained from HyClone Laboratories (Logan, UT). Recombinant murine sRANKL and macrophage-colony stimulating factor (M-CSF) were purchased from PeproTech (Rock Hill, NJ, USA). Tricarbonyldichlororuthenium (II) dimer (CORM-2), bilirubin, $FeCl_2$, and ruthenium chloride ($RuCl_3$) were purchased from Sigma-Aldrich (St Louis, MO, USA). All antibodies used in this study were purchased from Santa Cruz (Santa Cruz, CA, USA) or Cell Signaling (Beverly, MA, USA). Anti-NFATc1 monoclonal antibody was purchased from BD Biosciences Discovery Lab-ware (Bedford, MA).

Biochemical analyses

Whole cell lysates, cytosolic fractions, and nuclear extracts were prepared as previously described (23). After protein concentration was assessed by a BCA method, samples (30 mg protein) were electrophoresed on a 10% SDS-PAGE, and target protein levels were determined by Western blot analysis. Intracellular ROS accumulation was determined in RAW264.7 cells stimulated with 100 ng/ml RANKL in the presence or

absence of CORM-2 for 20 min using DCFH-DA, as previously described (24). ChIP assay was performed using cells stimulated with RANKL (100 ng/ml) in the presence or absence of CORM-2 (50 μM) for 2 h, as described in the manufacturer's instructions of Millipore ChIP Assay Kit (Millipore, Temecula, CA). Target sequences of NFATc1 promoter sequences were identified by PCR (30 cycles at 94°C for 1 min, 52°C for 1 min, 72°C for 30 s) using primer pairs spanning NFATc1-specific promoter regions containing NF-κB-binding sequence, forward 5′-ACGCCCATGCAATCT GTTAG-3′ and reverse 5′-TTGCATGCTGAAGTCATTATGT-3′, which amplify a 130 bp product. Products were identified on a 1.5% agarose gel. For reporter gene assay, RAW264.7 cells were transfected with 2 μg of NF-κB-Luc, NFATc1 promoter-Luc construct or in combination with each basic plasmid using Lipofectamine 3000. After 4 h incubation, cells were recovered in the fresh medium for 24 h, followed by incubation with RANKL (100 ng/ml) in the presence or absence of CORM-2 for 24 h. Reporter gene activity was assayed using a luciferase assay kit.

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CONFLICTS OF INTEREST

The authors have no conflicting financial interests.

REFERENCES

1. Asagiri M and Takayanagi H (2007) The molecular understanding of osteoclast differentiation. Bone 40, 251-264
2. Boyle WJ, Simonet WS and Lacey DL (2003) Osteoclast differentiation and activation. Nature 423, 337-342
3. Kobayashi N, Kadono Y, Naito A et al (2001) Segregation of TRAF6-mediated signalling pathways clarifies its role in osteoclastogenesis. EMBO J 20, 1271-1280
4. Walsh MC, Kim N, Kadono Y et al (2006) Osteoimmunology: interplay between the immune system and bone metabolism. Annu Rev Immunol 24, 33-63
5. Asagiri M, Sato K, Usami T, Ochi S, Nishina H and Yoshida H (2005) Autoamplification of NFATc1 expression determines its essential role in bone homeostasis. J Exp Med 202, 1261-1269
6. Ayer A, Zarjou A, Agarwal A and Stocker R (2016) Heme oxygenases in cardiovascular health and disease. Physiol Rev 96, 1449-1508
7. Kim YM, Pae HO, Park JE et al (2011) Heme oxygenase in the regulation of vascular biology: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal 14, 137-167
8. Van Phan T, Sul OJ, Ke K et al (2013) Carbon monoxide protects against ovariectomy-induced bone loss by inhibiting osteoclastogenesis. Biochem Pharmacol 85, 1145-1152
9. Tseng FJ, Chia WT, Wang CH et al (2015) Carbon monoxide inhibits receptor activator of NF-κB (RANKL)-induced osteoclastogenesis. Cell Physiol Biochem 36, 1250-1258
10. Ke K, Safder MA, Sul OJ et al (2015) Hemeoxygenase-1 maintains bone mass via attenuating a redox imbalance in osteoclast. Mol Cell Endocrinol 409, 11-20
11. Boyce BF (2013) Advances in the regulation of osteoclasts and osteoclast functions. J Dent Res 92, 860-867
12. Ryter SW, Alam J and Choi AM (2006) Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiol Rev 86, 583-650
13. Lee S, Lee SJ, Coronata AA et al (2014) Carbon monoxide confers protection in sepsis by enhancing beclin 1-dependent autophagy and phagocytosis. Antioxid Redox Signal 20, 432-442
14. Choi YK, Maki T, Mandeville ET et al (2016) Dual effects of carbon monoxide on pericytes and neurogenesis in traumatic brain injury. Nat Med 22, 1335-1341
15. Takayanagi H, Kim S, Koga T et al (2002) Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev Cell 3, 889-901
16. Santoro MG, Rossi A and Amici C (2003) NF-κB and virus infection: who controls whom. EMBO J 22, 2552-2560
17. Nakahira K, Kim HP, Geng XH et al (2006) Carbon monoxide differentially inhibits TLR signaling pathways by regulating ROS-induced trafficking of TLRs to lipid rafts. J Exp Med 203, 2377-2389
18. Chi PL, Liu CJ, Lee IT, Chen YW, Hsiao LD and Yang CM (2014) HO-1 induction by CO-RM2 attenuates TNF-α-induced cytosolic phospholipase A2 expression via inhibition of PKCα-dependent NADPH oxidase/ROS and NF-κB. Mediators Inflamm 2014, 279171
19. Lee NK, Choi YG, Baik JY et al (2005) A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. Blood 106, 852-859
20. Ha H, Kwak HB, Lee SW et al (2004) Reactive oxygen species mediate RANK signaling in osteoclasts. Exp Cell Res 301, 119-127
21. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN and Ames BN (1987) Bilirubin is an antioxidant of possible physiological importance. Science 235, 1043-1046
22. Yeh PY, Li CY, Hsieh CW, Yang YC, Yang PM and Wung BS (2014) CO-releasing molecules and increased heme oxygenase-1 induce protein S-glutathionylation to modulate NF-κB activity in endothelial cells. Free Radic Biol Med 70, 1-13
23. Kang N, Koo J, Wang S, Hur SJ and Bahk YY (2016) A systematic study of nuclear interactome of C-terminal domain small phosphatase-like 2 using inducible expression system and shotgun proteomics. BMB Rep 49, 319-324
24. Park GS, Kim JK and Kim JH (2016) Anti-inflammatory action of ethanolic extract of Ramulus mori on the BLT2-linked cascade. BMB Rep 49, 232-237