

# Honokiol stimulates osteoblastogenesis by suppressing NF- $\kappa$ B activation

MASAYOSHI YAMAGUCHI<sup>2\*</sup>, JACK L. ARBISER<sup>1,3,4</sup> and M. NEALE WEITZMANN<sup>1,2,4\*</sup>

<sup>1</sup>Atlanta Department of Veterans Affairs Medical Center, Decatur, GA 30033; <sup>2</sup>Division of Endocrinology and Metabolism and Lipids, Department of Medicine, <sup>3</sup>Department of Dermatology and <sup>4</sup>Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA 30322, USA

Received April 27, 2011; Accepted May 30, 2011

DOI: 10.3892/ijmm.2011.786

**Abstract.** *Magnolia officinalis*, a component of Asian herbal teas, has long been employed in traditional Japanese and Chinese medicine to treat numerous maladies. Honokiol, a biphenolic compound, is now considered to be one of the major active ingredients of *Magnolia* extract, and is under intense investigation for its anti-angiogenic, anti-inflammatory, anti-tumor and neuroprotective properties. Biochemically, honokiol has been recognized to modulate the nuclear factor  $\kappa$  B (NF- $\kappa$ B) signal transduction pathway suggesting that it possesses anti-inflammatory properties. Inflammation is intimately associated with bone turnover and skeletal deterioration and consequently, anti-inflammatory drugs may hold significant promise as bone protective agents to stem bone loss in osteoporotic conditions. We and others have demonstrated that suppression of NF- $\kappa$ B blunts osteoclastic bone resorption, but promotes osteoblastic bone formation. Indeed previous studies have demonstrated the anti-osteoclastogenic effects of honokiol, however, activities on osteoblast differentiation and activity have yet to be investigated. In this study, we show that honokiol is a potent inducer of *in vitro* osteoblast differentiation by virtue of its capacity to suppress basal and tumor necrosis factor alpha (TNF $\alpha$ )-induced NF- $\kappa$ B activation and to alleviate the suppressive action of TNF $\alpha$  on bone morphogenetic protein (BMP)-2-induced Smad activation. Our data confirm that honokiol may have considerable promise as a dual anabolic/anti-catabolic agent for the amelioration of multiple osteoporotic diseases.

## Introduction

Skeletal renewal is a delicate balance between bone resorbing osteoclasts and bone forming osteoblasts. Osteoclasts form from precursors that circulate among the monocyte lineage and differentiate into osteoclasts under the influence of the key osteoclastogenic cytokine receptor activator of nuclear factor  $\kappa$  B (NF- $\kappa$ B) ligand (RANKL) (1,2). Activation of the NF- $\kappa$ B signal transduction pathway by RANKL is an essential step in osteoclast differentiation and function (3) and pharmacological antagonists of NF- $\kappa$ B activation repress osteoclastogenesis *in vitro* (4-7) and bone resorption *in vivo* (8,9). By contrast, activation of NF- $\kappa$ B signaling by inflammatory cytokines such as TNF $\alpha$  potentially inhibits osteoblast differentiation and function *in vitro* (5,10-13) and *in vivo* (10,14,15). By contrast, suppression of NF- $\kappa$ B promotes osteoblast differentiation and mineralization *in vitro* (10) and bone formation at baseline (16) and in *in vivo* models of estrogen deficiency (16,17) and of fracture repair (18,19).

Honokiol is a small-molecule polyphenol isolated from the genus *Magnolia* and has been found to have anti-angiogenic, anti-inflammatory, and anti-tumor properties in preclinical models, with low toxicity (20). Biochemically, honokiol has been recognized to regulate NF- $\kappa$ B signaling and to block TNF $\alpha$ -induced NF- $\kappa$ B activation, I $\kappa$ B $\alpha$  phosphorylation, and I $\kappa$ B $\alpha$  degradation (20).

Consistent with this anti-NF- $\kappa$ B activity honokiol was found to inhibit RANKL-induced osteoclastogenesis *in vitro* (21) and to inhibit the progression of collagen-induced arthritis (an animal model of the inflammatory autoimmune disease, rheumatoid arthritis) *in vivo*, by down-regulating inflammatory cytokines, matrix metalloproteinases and blocking oxidative tissue damage (22).

Given the capacity of honokiol to antagonize NF- $\kappa$ B activation we examined whether honokiol could promote osteoblast differentiation and mineralization *in vitro*. Our data confirm this notion and suggest that honokiol may indeed have significant potential for use as a bone anabolic agent.

## Material and methods

**Materials.**  $\alpha$ -minimal essential medium ( $\alpha$ -MEM) and antibiotics (penicillin and streptomycin) were purchased from

Correspondence to: Dr M. Neale Weitzmann, Emory University School of Medicine, 101 Woodruff Circle, 1305 WMRB, Atlanta, GA 30322, USA

E-mail: mweitzm@emory.edu

Dr Masayoshi Yamaguchi, Emory University School of Medicine, 101 Woodruff Circle, 1329 WMRB, Atlanta, GA 30322, USA

E-mail: yamamasa1155@yahoo.co.jp

\*Contributed equally

**Key words:** honokiol, nuclear factor  $\kappa$ B, osteoblast, osteoporosis

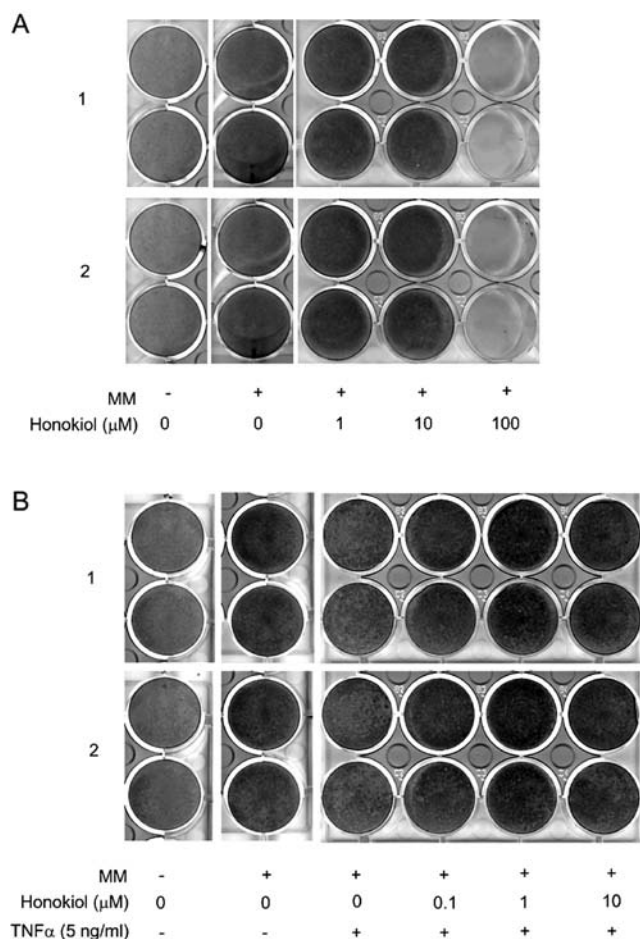


Figure 1. Honokiol promotes osteoblast differentiation and mineralization and alleviates the suppression of osteoblast mineralization induced by TNF $\alpha$  *in vitro*. (A) MC3T3 cells were cultured in the presence (+) or absence (-) of mineralizing medium (MM) with a dose range of honokiol (0, 1, 10 or 100  $\mu$ M), and (B) with or without TNF $\alpha$  (5 ng/ml). Cultures were stained with Alizarin Red-S at 18 days for visualization of calcium deposition. Two sets of data-labeled (1 and 2) are shown and represent independent experiments, with each data point performed in duplicate. All wells within each experiment were from the same plate but were digitally separated and reorganized for clarity. Non-contiguous wells are separated by a white space to indicate this fact.

Invitrogen Corp. (Carlsbad, CA). Fetal bovine serum (FBS) was from Hyclone (Logan, UT). TNF $\alpha$ , transforming growth factor beta (TGF $\beta$ ) and BMP-2 were from R&D Systems (Minneapolis, MN). Antibodies for Western blot analysis were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Honokiol and all other reagents were purchased from the Sigma-Aldrich Chemical Corp., (St. Louis, MO) unless otherwise specified.

**Cell culture.** The pre-osteoblastic cell line MC3T3-E1, clone 14 (MC3T3) was purchased from the American Type Culture Collection (Manassas, VA) and cultured as previously described (10,23).

**Osteoblast differentiation assays and Alizarin Red-S staining.** MC3T3 cells, were plated and cultured for 72 h in  $\alpha$ -MEM (1.0 ml/well) containing 10% FBS in 12-well dishes at a density of ( $1.0 \times 10^5$  cells/well). The medium was aspirated and changed to mineralization medium ( $\alpha$ -MEM supplemented with 10% FBS, L-ascorbic acid (100  $\mu$ g/ml) and

4 mM  $\beta$ -glycerophosphate) as previously described (5,10,24). Honokiol was added at the indicated dose and cells were replenished with fresh medium every 3 days. At 18 days, cells were rinsed with PBS and mineralization nodules were visualized by fixing the cells in 75% ethanol for 30 min at 4°C followed by staining with Alizarin Red-S (40 mM, pH 6.2) for 30 min at room temperature. Excess stain was removed by copious washing with distilled water. Plates were imaged using a flatbed scanner (Epson Perfection V600 Photo).

**NF- $\kappa$ B constructs and luciferase assays.** The NF- $\kappa$ B responsive reporter pNF- $\kappa$ B-Luc (BD Biosciences) or pGL3-Smad, responsive to all R-Smads, was used as previously described by us (5,10). Briefly, reporter plasmid was transfected into MC3T3 cells ( $1 \times 10^5$  cells/well) using Lipofectamine 2000 reagent (Invitrogen) in  $\alpha$ -MEM without FBS and antibiotics. Five hours later the medium was changed to  $\alpha$ -MEM containing 10% FBS plus antibiotics and cells were treated with TNF $\alpha$  to stimulate NF- $\kappa$ B activity. Parallel groups received honokiol at the indicated dose. Cells were extracted with passive lysis buffer (Promega Corp., Madison, WI) 24 h later, and luciferase activity was measured using the Luciferase Assay System of Promega (Madison, WI), on a microplate luminometer (Turner Designs, Sunnyvale, CA, USA).

**Western blot analysis.** Cells were plated in 35 mm wells at a density of  $10^6$  cells/well in 2 ml of medium ( $\alpha$ -MEM + 10% FBS and antibiotics) and cultured for 24 h prior to addition of honokiol (10  $\mu$ M) for an additional 24 h, followed by addition of TNF $\alpha$  (10 ng/ml) for 60 min. Cultures were subsequently lysed for preparation of cytosolic and nuclear extracts as previously described (25) for Western blotting using antibodies against p65, and I $\kappa$ B $\alpha$ . Loading controls consisted of  $\beta$ -actin for cytosolic proteins, or proliferating cell nuclear antigen (PCNA) for nuclear proteins.

**Statistical analysis.** Statistical significance was determined using GraphPad InStat version 3 for Windows XP (GraphPad Software, Inc., La Jolla, CA). Multiple comparisons were performed by one-way analysis of variance (ANOVA) with the Tukey-Kramer multiple comparisons post-test for parametric data. Gaussian distribution was assessed using the Kolmogorov and Smirnov test. P-value <0.05 was considered statistically significant.

## Results

**Honokiol augments the differentiation of MC3T3 cells into mineralizing osteoblasts and alleviates the suppressive effect of TNF $\alpha$  on osteoblast mineralization.** Honokiol has been reported to suppress NF- $\kappa$ B in some cell systems suggesting that it may possess bone anabolic activity. To investigate the effect of honokiol on *in vitro* osteoblast differentiation and activity we treated MC3T3 cells with honokiol (1, 10 or 100  $\mu$ M) in mineralizing medium for 18 days and stained for calcium deposition with Alizarin Red-S. The data show (Fig. 1A) that at 1 and 10  $\mu$ M honokiol augmented the differentiation of MC3T3 cells into mineralizing osteoblasts. At 100  $\mu$ M mineralization was inhibited, although cells were still alive and attached to the plate.

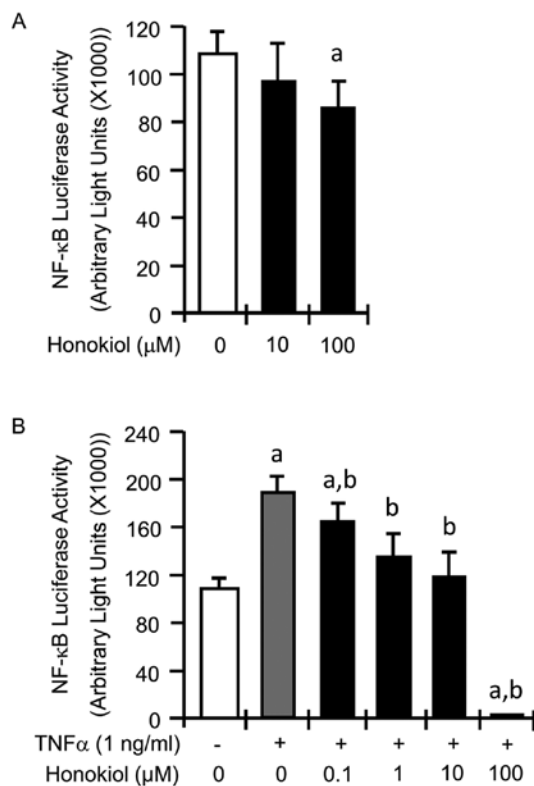


Figure 2. Honokiol suppresses NF- $\kappa$ B activation in osteoblast precursors. (A) MC3T3 cells were transfected with pNF- $\kappa$ B-Luc, an NF- $\kappa$ B activity reporter plasmid and cells were treated with honokiol (10 or 100  $\mu$ M). <sup>a</sup>P<0.01 vs. control (white bar). (B) MC3T3 cells were transfected with pNF- $\kappa$ B-Luc, and cells were treated with honokiol (0.1, 1, 10 or 100  $\mu$ M) in the presence or absence of TNF $\alpha$  (1 ng/ml). <sup>a</sup>P<0.001 vs. control (white bar); <sup>b</sup>P<0.001 vs. TNF only (grey bar). Data are presented as the mean  $\pm$  SD of 5 replicate wells per data point and are representative of three independent experiments.

TNF $\alpha$  is an established inhibitor of osteoblastic differentiation both *in vivo* and *in vitro* (10,26). Consistent with these known actions, TNF $\alpha$  (5 ng/ml) suppressed mineralization of MC3T3 cells. Addition of honokiol at 0.1  $\mu$ M and above alleviated the repressive effect of TNF $\alpha$  and potentiated mineralization (Fig. 1B).

**Honokiol suppresses basal and TNF $\alpha$ -induced NF- $\kappa$ B activity in MC3T3 cells.** Because honokiol is reported to antagonize NF- $\kappa$ B, a pathway inhibitory to osteoblast differentiation and a key mechanism of TNF $\alpha$  signaling we next investigated whether honokiol antagonizes basal and/or TNF $\alpha$ -induced NF- $\kappa$ B activation. We transiently transfected MC3T3 cells with an NF- $\kappa$ B luciferase reporter and quantitated luciferase activity in the presence or absence of honokiol. Honokiol weakly, but significantly, suppressed basal NF- $\kappa$ B activity (Fig. 2A) and potently and dose-dependently (0.1 to 100  $\mu$ M) suppressed TNF $\alpha$ -induced NF- $\kappa$ B activity (Fig. 2B).

**Honokiol antagonizes the nuclear accumulation of p65 NF- $\kappa$ B subunit by TNF $\alpha$  and promotes cytosolic retention of I $\kappa$ B $\alpha$ .** As the NF- $\kappa$ B subunit p65 is considered to be a major species in osteoblasts (26) we performed Western blot analyses for p65 on nuclear and cytosolic extracts from MC3T3 osteoblast precursors, in the presence or absence of TNF $\alpha$  and/or honokiol.

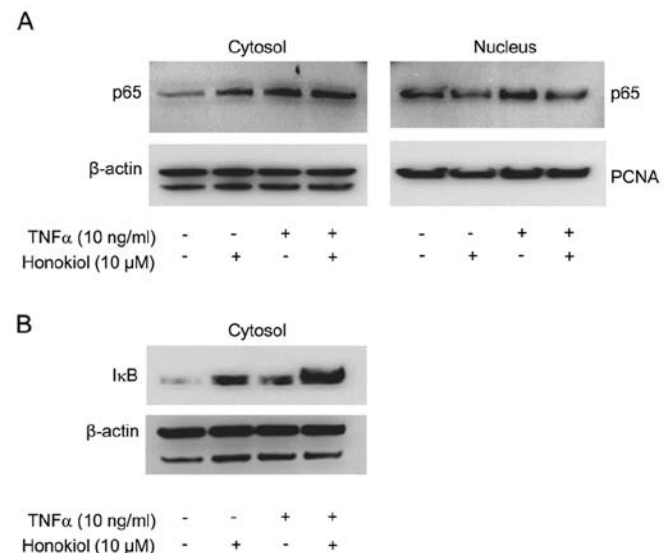


Figure 3. Honokiol suppresses nuclear accumulation of p65 in the nucleus and promotes I $\kappa$ B $\alpha$  retention in the cytosol of osteoblast precursors. MC3T3 cells were treated with honokiol (10  $\mu$ M) for 18 h followed by addition of TNF $\alpha$  (10 ng/ml) for 60 min. Cells were lysed and cytosolic and nuclear proteins extracted and Western blot analyses performed for (A) the NF- $\kappa$ B subunit p65 or (B) I $\kappa$ B $\alpha$ .  $\beta$ -actin was used as a cytosolic loading control and PCNA as a nuclear loading control. Data are representative of two independent experiments.

TNF $\alpha$ -induced a pooling of p65 in the nucleus (Fig. 3A) while honokiol markedly reduced basal and TNF $\alpha$ -induced p65 nuclear translocation. Consistent with these data honokiol promoted cytosolic retention of I $\kappa$ B $\alpha$  under basal conditions as well as following TNF $\alpha$  stimulation (Fig. 3B).

**Honokiol alleviates the suppressive actions of TNF $\alpha$  on BMP-2- and TGF $\beta$ -induced Smad activation in MC3T3 cells.** TNF $\alpha$  suppresses osteoblast differentiation in part by antagonizing Smad activation by bone morphogenetic proteins (BMP) including BMP-2 and early osteoblast commitment factor TGF $\beta$ . We thus examined the effect of honokiol on basal, BMP-2-induced and TGF $\beta$ -induced Smad activation and the capacity of honokiol to reverse the suppressive action of TNF $\alpha$  on Smad induction by BMP-2 and TGF $\beta$  using a Smad-luciferase reporter responsive to all Smad species (10). While honokiol had no direct effect on basal, BMP-2- (Fig. 4A), or TGF $\beta$ -induced (Fig. 5A) Smad-activation, it significantly reversed the suppressive activity of TNF $\alpha$  on BMP-2 (Fig. 4B) and TGF $\beta$  (Fig. 5B) induced Smad activation.

## Discussion

Honokiol has been recognized as an antagonist of NF- $\kappa$ B activation in a number of cell types, and consistent with such action has been reported to inhibit osteoclast differentiation (21), an NF- $\kappa$ B-dependent process (7,8,27,28). By contrast, we have previously demonstrated that TNF $\alpha$ , a potent inducer of NF- $\kappa$ B, is a suppressor of bone formation *in vivo* in mice and even at physiological concentrations significantly lowers peak attainable bone mineral density (BMD) (10).

The reverse process however, NF- $\kappa$ B suppression, promotes osteoblast differentiation *in vitro* by pharmaco-

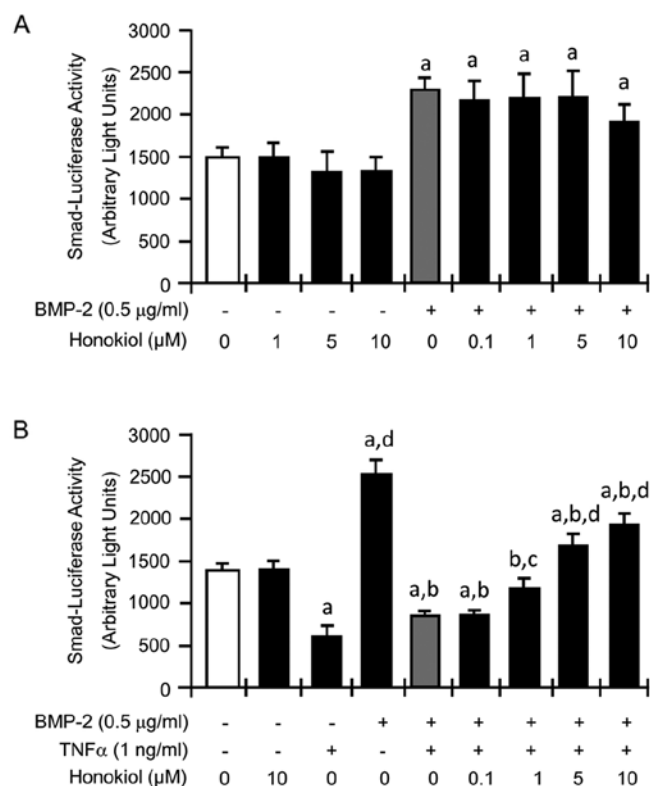


Figure 4. Honokiol alleviates TNF $\alpha$ -induced suppression of BMP-2-induced Smad activation. (A) MC3T3 cells were transfected with pGL3-Smad luciferase reporter plasmid, and the cells were treated with BMP-2 (0.5  $\mu$ g/ml) or vehicle in the presence or absence of honokiol (0.1, 1, 5 or 10  $\mu$ M). \* $P$ <0.001 vs. control (white bar). (B) MC3T3 cells were transfected with pGL3-Smad reporter plasmid, and the cells were treated with BMP-2 (0.5  $\mu$ g/ml) or vehicle in the presence or absence of honokiol (0.1, 1, 5 or 10  $\mu$ M) and/or TNF $\alpha$  (1 ng/ml). <sup>a</sup> $P$ <0.001 vs. control (white bar); <sup>b</sup> $P$ <0.001 vs. BMP-2 only; <sup>c</sup> $P$ <0.01 vs. BMP-2 + TNF $\alpha$  (grey bar) and <sup>d</sup> $P$ <0.001 vs. BMP-2 + TNF $\alpha$  (grey bar). Data are presented as the mean  $\pm$  SD of 5 replicate wells per data point and are representative of two independent experiments.

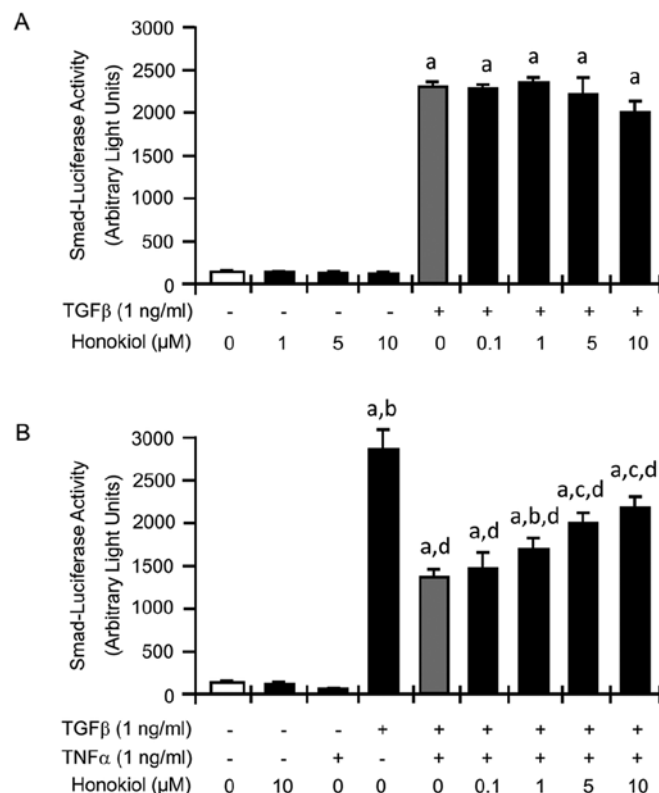


Figure 5. Honokiol alleviates TNF $\alpha$ -induced suppression of TGF $\beta$ -induced Smad activation. (A) MC3T3 cells were transfected with pGL3-Smad luciferase reporter plasmid, and cells treated with TGF $\beta$  (1 ng/ml) or vehicle, in the presence or absence of honokiol (0.1, 1, 5 or 10  $\mu$ M). \* $P$ <0.001 vs. control (white bar). (B) MC3T3 cells were transfected with pGL3-Smad reporter plasmid, and cells were treated with TGF $\beta$  (1 ng/ml) or vehicle, in the presence or absence of honokiol (0.1, 1, 5 or 10  $\mu$ M) and/or TNF $\alpha$  (1 ng/ml). \* $P$ <0.001 vs. control (white bar); <sup>b</sup> $P$ <0.01; <sup>c</sup> $P$ <0.001 vs. TGF $\beta$  + TNF $\alpha$  (grey bar); <sup>d</sup> $P$ <0.001 vs. BMP-2 only. Data are presented as the mean  $\pm$  SD of 5 replicate wells per data point and are representative of two independent experiments.

logical antagonists (10) as well as by nutritional factors with bone anabolic properties including vitamin K2 (5), and the carotenoids p-hydroxycinnamic acid and  $\beta$ -cryptoxanthin (11). Based on the actions of these other NF- $\kappa$ B antagonists, we hypothesized that honokiol by virtue of its anti-NF- $\kappa$ B activity may also possess bone anabolic activity. We examined this notion *in vitro*, and found that honokiol does indeed up-regulate mineralization in the pre-osteoblastic cell line MC3T3, as well as reverses the suppressive action of TNF $\alpha$  on mineralization. Honokiol suppressed NF- $\kappa$ B activation in the context of a biological assay (the NF- $\kappa$ B-driven reporter system) demonstrating a potent and sustained effect. Consistent with the bio-assay we found that nuclear translocation of the NF- $\kappa$ B subunit p65 was suppressed by honokiol and that the cytosolic concentration of the physiological NF- $\kappa$ B activation inhibitor I $\kappa$ B, was intensified following treatment by honokiol. Our data are consistent with previous findings showing that honokiol inhibits TNF-induced NF- $\kappa$ B activation, and I $\kappa$ B degradation (20).

TGF $\beta$  is an early osteoblast commitment factor (29) and induces migration of bone mesenchymal stem cells (osteoblast progenitors) to sites of bone turnover (30). BMPs by contrast, are potent inducers of osteoblast differentiation (31). Both TGF $\beta$  and BMPs are potent inducers of Smad signaling,

although TGF $\beta$  uses predominantly R-Smads 2 and 3, while BMPs promote mainly R-Smads 1, 5 and 8. We previously reported that one mechanism by which TNF $\alpha$  suppresses osteoblast differentiation is through NF- $\kappa$ B-mediated antagonism of TGF $\beta$ - and BMP-2-induced Smad signaling in differentiating osteoblasts (10). Other studies have further reported that NF- $\kappa$ B signaling in osteoblasts up-regulates the inhibitor of Smad-signaling, Smad7 (32), and that TNF $\alpha$  promotes proteasomal degradation of BMP signaling proteins by up-regulating Smad ubiquitination regulatory factor 1 (Smurf1), a process that leads to systemic bone loss (15). We recently demonstrated that NF- $\kappa$ B antagonists including a pharmacological peptide based on the Nemo Binding Domain (10) and vitamin K2 (5) can alleviate this repressive action of TNF $\alpha$  on Smad signaling. Our data now reveal that honokiol also possesses this ability.

Although, anti-resorptive agents have long been the preferred standard of care for the amelioration of bone loss, because the processes of bone resorption and bone formation are tightly 'coupled', anti-resorptive therapies are also observed clinically to simultaneously suppress bone formation (33) leading to a 'low bone turnover state'. Although in general anti-resorptive agents do an excellent job of preventing additional bone loss, they do not allow for adequate regeneration of lost

bone mass. In recent years an intensive effort has begun to identify or develop anabolic agents capable of rebuilding lost bone mineral density. At present teriparatide, a fragment of the human parathyroid hormone is the only Food and Drug Administration (FDA)-approved anabolic agent currently available. This agent represents a significant leap forward, but as a biologic-based agent its use is limited by high cost and the need for daily injection. Furthermore, therapy is not recommended for more than 2 years due to the potential for osteosarcoma (34). As a consequence there is intense interest for the identification of additional anabolic agents. We have previously suggested that NF- $\kappa$ B antagonists may represent novel dual anabolic and anti-catabolic drugs (10). Our data suggest that honokiol may represent one such agent. A significant advantage of honokiol over biologics that require injection is its demonstrated bioavailability following oral delivery in animal models (20). Furthermore, as honokiol is a simple polyphenol compound, it can be synthesized in large concentrations at low cost.

In conclusion, our data suggest that honokiol may have significant potential for development into a bone anabolic agent for amelioration of bone loss in multiple metabolic disease states. Studies in animal models are now needed to demonstrate bone anabolic activity of honokiol *in vivo*.

## References

- Teitelbaum SL: Bone resorption by osteoclasts. *Science* 289: 1504-1508, 2000.
- Khosla S: Minireview: the OPG/RANKL/RANK system. *Endocrinology* 142: 5050-5055, 2001.
- Boyce BF, Yao Z and Xing L: Functions of nuclear factor kappaB in bone. *Ann NY Acad Sci* 1192: 367-375, 2010.
- Hall TJ, Schaeublin M, Jeker H, Fuller K and Chambers TJ: The role of reactive oxygen intermediates in osteoclastic bone resorption. *Biochem Biophys Res Commun* 207: 280-287, 1995.
- Yamaguchi M and Weitzmann MN: Vitamin K2 stimulates osteoblastogenesis and suppresses osteoclastogenesis by suppressing NF- $\kappa$ B activation. *Int J Mol Med* 27: 3-14, 2011.
- Feng R, Anderson G, Xiao G, *et al*: SDX-308, a nonsteroidal anti-inflammatory agent, inhibits NF-kappaB activity, resulting in strong inhibition of osteoclast formation/activity and multiple myeloma cell growth. *Blood* 109: 2130-2138, 2007.
- Clohisy JC, Hirayama T, Frazier E, Han SK and Abu-Amer Y: NF- $\kappa$ B signaling blockade abolishes implant particle-induced osteoclastogenesis. *J Orthop Res* 22: 13-20, 2004.
- Strait K, Li Y, Dillehay DL and Weitzmann MN: Suppression of NF- $\kappa$ B activation blocks osteoclastic bone resorption during estrogen deficiency. *Int J Mol Med* 21: 521-525, 2008.
- Dai S, Hirayama T, Abbas S and Abu-Amer Y: The IkappaB kinase (IKK) inhibitor, NEMO-binding domain peptide, blocks osteoclastogenesis and bone erosion in inflammatory arthritis. *J Biol Chem* 279: 37219-37222, 2004.
- Li Y, Li A, Strait K, Zhang H, Nanes MS and Weitzmann MN: Endogenous TNFalpha lowers maximum peak bone mass and inhibits osteoblastic smad activation, through NF-kappaB. *J Bone Miner Res* 22: 646-655, 2007.
- Yamaguchi M and Weitzmann MN: The bone anabolic carotenoids p-hydroxycinnamic acid and beta-cryptoxanthin antagonize NF-kappaB activation in MC3T3 preosteoblasts. *Mol Med Rep* 2: 641-644, 2009.
- Yamaguchi M and Weitzmann MN: The estrogen 17beta-estradiol and phytoestrogen genistein mediate differential effects on osteoblastic NF-kappaB activity. *Int J Mol Med* 23: 297-301, 2009.
- Gilbert L, He X, Farmer P, *et al*: Expression of the osteoblast differentiation factor RUNX2 (Cbfa1/AML3/Pebsp2alpha A) is inhibited by tumor necrosis factor-alpha. *J Biol Chem* 277: 2695-2701, 2002.
- Kaneki H, Guo R, Chen D, *et al*: Tumor necrosis factor promotes Runx2 degradation through up-regulation of Smurf1 and Smurf2 in osteoblasts. *J Biol Chem* 281: 4326-4333, 2006.
- Guo R, Yamashita M, Zhang Q, *et al*: Ubiquitin ligase Smurf1 mediates tumor necrosis factor-induced systemic bone loss by promoting proteasomal degradation of bone morphogenetic signaling proteins. *J Biol Chem* 283: 23084-23092, 2008.
- Chang J, Wang Z, Tang E, *et al*: Inhibition of osteoblastic bone formation by nuclear factor-kappaB. *Nat Med* 15: 682-689, 2009.
- Alles N, Soysa NS, Hayashi J, *et al*: Suppression of NF-kappaB increases bone formation and ameliorates osteopenia in ovariectomized mice. *Endocrinology* 151: 4626-4634, 2010.
- Wahl EC, Aronson J, Liu L, *et al*: Restoration of regenerative osteoblastogenesis in aged mice: modulation of TNF. *J Bone Miner Res* 25: 114-123, 2010.
- Wahl EC, Aronson J, Liu L, *et al*: Direct bone formation during distraction osteogenesis does not require TNFalpha receptors and elevated serum TNFalpha fails to inhibit bone formation in TNFR1 deficient mice. *Bone* 46: 410-417, 2010.
- Fried LE and Arbiser JL: Honokiol, a multifunctional antiangiogenic and antitumor agent. *Antioxid Redox Signal* 11: 1139-1148, 2009.
- Ahn KS, Sethi G, Shishodia S, Sung B, Arbiser JL and Aggarwal BB: Honokiol potentiates apoptosis, suppresses osteoclastogenesis, and inhibits invasion through modulation of nuclear factor-kappaB activation pathway. *Mol Cancer Res* 4: 621-633, 2006.
- Kim KR, Park KK, Chun KS and Chung WY: Honokiol inhibits the progression of collagen-induced arthritis by reducing levels of pro-inflammatory cytokines and matrix metalloproteinases and blocking oxidative tissue damage. *J Pharmacol Sci* 114: 69-78, 2010.
- Yamaguchi M and Weitzmann MN: The bone anabolic carotenoid beta-cryptoxanthin enhances transforming growth factor-beta1-induced SMAD activation in MC3T3 preosteoblasts. *Int J Mol Med* 24: 671-675, 2009.
- Sugimoto E and Yamaguchi M: Anabolic effect of genistein in osteoblastic MC3T3-E1 cells. *Int J Mol Med* 5: 515-520, 2000.
- Andrews NC and Faller DV: A rapid microprep preparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. *Nucleic Acids Res* 19: 2499, 1991.
- Nanes MS: Tumor necrosis factor-alpha: molecular and cellular mechanisms in skeletal pathology. *Gene* 321: 1-15, 2003.
- Xing L, Bushnell TP, Carlson L, *et al*: NF-kappaB p50 and p52 expression is not required for RANK-expressing osteoclast progenitor formation but is essential for RANK- and cytokine-mediated osteoclastogenesis. *J Bone Miner Res* 17: 1200-1210, 2002.
- Novack DV, Yin L, Hagen-Stapleton A, *et al*: The IkappaB function of NF-kappaB2 p100 controls stimulated osteoclastogenesis. *J Exp Med* 198: 771-781, 2003.
- Janssens K, Ten Dijke P, Janssens S and Van Hul W: Transforming growth factor-beta1 to the bone. *Endocr Rev* 26: 743-774, 2005.
- Tang Y, Wu X, Lei W, *et al*: TGF-beta1-induced migration of bone mesenchymal stem cells couples bone resorption with formation. *Nat Med* 15: 757-765, 2009.
- Wan M and Cao X: BMP signaling in skeletal development. *Biochem Biophys Res Commun* 328: 651-657, 2005.
- Elisei RA, Schwarz EM, Zuscik MJ, O'Keefe RJ, Drissi H and Rosier RN: Smad7 mediates inhibition of Saos2 osteosarcoma cell differentiation by NFkappaB. *Exp Cell Res* 312: 40-50, 2006.
- McClung MR, Lewiecki EM, Cohen SB, *et al*: Denosumab in postmenopausal women with low bone mineral density. *N Engl J Med* 354: 821-831, 2006.
- Hodsman AB, Bauer DC, Dempster DW, *et al*: Parathyroid hormone and teriparatide for the treatment of osteoporosis: a review of the evidence and suggested guidelines for its use. *Endocr Rev* 26: 688-703, 2005.