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Mangiferin Attenuates Osteoclastogenesis, Bone Resorption, and RANKL-Induced Activation of NF-κB and ERK

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ABSTRACT

Osteolytic bone diseases such as osteoporosis have a common pathological feature in which osteoclastic bone resorption outstrips bone synthesis. Osteoclast formation and activation are regulated by receptor activator of nuclear factor κB ligand (RANKL). The induction of RANKL-signaling pathways occurs following the interaction of RANKL to its cognate receptor, RANK. This specific binding drives the activation of downstream signaling pathways; which ultimately induce the formation and activation of osteoclasts. In this study, we showed that a natural immunomodulator, mangiferin, inhibits osteoclast formation and bone resorption by attenuating RANKL-induced signaling. Mangiferin diminished the expression of osteoclast marker genes, including cathepsin K, calcitonin receptor, DC-STAMP, and V-ATPase d2. Mechanistic studies revealed that mangiferin inhibits RANKL-induced activation of NF-κB, concomitant with the inhibition of IκB-α degradation, and p65 nuclear translocation. In addition, mangiferin also exhibited an inhibitory effect on RANKL-induced ERK phosphorylation. Collectively, our data demonstrates that mangiferin exhibits anti-resorptive properties, suggesting the potential application of mangiferin for the treatment and prevention of bone diseases involving excessive osteoclastic bone resorption. J. Cell. Biochem. 112: 89–97, 2011.

KEY WORDS: MANGIFERIN; OSTEOCLASTOGENESIS; BONE RESORPTION; RANKL; NF-κB

Bone diseases including osteoporosis, Paget's disease, bone metastatic diseases, erosive arthritis, aseptic bone loosening, and non-union of fractures are all characterized by similar pathological features, whereby osteoclastic bone resorption outstrips bone synthesis. A recent consensus statement by the National Institute of Health (NIH) indicates that osteoporosis is a devastating disorder with significant physical, psychosocial, and financial consequences. Osteoporotic hip fractures in the elderly are the most serious consequence of osteoporosis and is correlated with increased mortality rate [Iacovino, 2001; Cummings and Melton, 2002]. Despite current treatments available, the number of osteoporosis related fractures is still expected to increase exponentially in our aging population during the next century [Fitzpatrick, 2003]. By understanding the nature of the formation and function of osteoclasts, the use of natural osteoclast inhibitors may provide a novel way to treat osteolytic conditions.

Multinucleated osteoclasts of the hematopoietic stem cell origin are the principal cells responsible for bone resorption [Teitelbaum, 2000]. This unique feature of the osteoclast is the main target of current anti-resorptive drugs. Receptor activator of nuclear factor-kappa B (NF-κB) ligand (RANKL) is the key cytokine obligatory for osteoclast formation and activation [Lacey et al., 1998]. The interaction between RANKL and its cognate receptor RANK results in a cascade of intracellular signaling events including NF-κB, Akt,

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mitogen-activated protein kinases (MAPK), nuclear factor of activated T cells (NFAT), ionic calcium, and calcium/calmodulin-dependent kinase. Among these pathways, NF-κB signaling plays an indispensible role in osteoclastogenesis [Franzoso et al., 1997; Iotsova et al., 1997; Xu et al., 2009].

Mangiferin, a naturally occurring polyphenol commonly found in both mango and papaya [Wilkinson et al., 2008] exhibits anti-tumor, antiviral, anti-diabetic, antioxidant, and anti-apoptotic properties [Rajendran et al., 2008; Campos-Esparza et al., 2009]. A study by Sarkar et al. [2004] showed that mangiferin blocks tumor necrosis factor (TNF)-induced NF-κB activation. Osteolytic conditions, including periprosthetic osteolysis, arthritis, Paget's disease of bone, and periodontitis are often associated with the over-activation of NF-κB signaling in osteoclasts resulting in excessive osteoclastic activity [Xu et al., 2009]. The anti-NF-κB properties of mangiferin may prove itself as a potential alternative medicine for the treatment of osteolytic bone diseases.

Given that mangiferin has been used in traditional folk Chinese medicine, there is a growing need for evidence- and mechanistic-based study to facilitate the use of mangiferin in the prevention and treatment of osteolytic bone diseases.

MATERIALS AND METHODS

MEDIA AND REAGENTS

RAW264.7 cells were obtained from the American Type Culture Collection (Rockville, MD). Alpha modified of Eagles Medium (α-MEM) and fetal bovine serum (FBS) was purchased from TRACE (Sydney, Australia). Mangiferin was purchased from Sigma–Aldrich (Sydney, Australia) and dissolved in Dimethyl sulfoxide (DMSO). The luciferase assay system was obtained from Promega (Sydney, Australia). Recombinant GST-rRANKL protein was expressed and purified as previously described [Xu et al., 2000].

IN VITRO OSTEOCLASTOGENESIS ASSAY

Freshly isolated bone marrow macrophages (BMM) from C57/BL6 mice were cultured with macrophage-colony stimulating factor (M-CSF, 10 ng/ml) for the first 3 days. BMM were then seeded onto a 96-well plate (8×10^3 cells/well) with medium containing M-CSF (10 ng/ml) and in the presence or absence of RANKL (100 ng/ml). Medium was replaced every 2–3 days and after 7 days, cultures were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min at room temperature and then washed four times with 1× PBS. Fixed cells were stained for tartrate-resistant acid phosphatase (TRACP) using the Diagnostic Acid Phosphatase kit (Sigma) according to the manufacturer's instructions and TRACP positive multi-nucleated cells (>3 nuclei) were scored as osteoclast-like (OCL) cells.

IMMUNOFLUORESCENT STAINING AND CONFOCAL MICROSCOPY

For immunofluorescence studies, 1×10^4 RAW264.7 cells were seeded onto a 6-well plate pre-seeded with five coverslips. After overnight of incubation to allow for cell attachment, cells were stimulated with RANKL (100 ng/ml) for 5 days to induce osteoclast formation in the absence or presence of mangiferin (0.1 and 0.5 mM). After incubation, cells were washed twice with 1× PBS, fixed for 15 mins at room temperature with 4% paraformaldehyde in 1× PBS (pH 7.4) and then washed extensively with 1× PBS. Cells were then permeabilized in 1× PBS containing 0.1% Triton X-100, washed twice in 1× PBS containing 0.2% BSA (0.2% BSA–PBS). Cells were then labeled for 45 min at room temperature with an Alexa Fluor 488 phallotoxin (Molecular Probes, Inc., Eugene, OR) diluted 1:500 in 0.2% BSA–PBS. Cells were then washed in 0.2% BSA–PBS and PBS as above, counter stained for 3 min at room temperature with DAPI (Santa Cruz Biotechnology, Inc., CA) and mounted for confocal microscopy (MRC-1000; Bio-Rad, CA) as previously described [Ang et al., 2009].

BONE RESORPTION PIT ASSAY

Human giant cell tumor of bone (GCT) of bone samples was freshly isolated from patients who have undergone surgery at Sir Charles Gairdner Hospital (Nedlands, WA, Australia). Tumor tissues were finely chopped in complete medium and the resultant cell suspension was passed through a 100 μm nylon cell strainer (BD Bioscience, MA), and approximately 200 OCL cells were seeded onto bovine bone slices in the presence and absence of mangiferin. After culturing for 24 h at 37°C incubation, bovine bone slices were incubated for 2 h in 2 M NaOH and OCLs were removed by mechanical agitation and sonication. Resorption pits were visualized under Philips XL30 scanning electron microscope (SEM) and the percentage of bone surface area resorbed was quantified using Scion Image software (Scion Cooperation, National Institutes of Health) [Yip et al., 2006].

RNA ISOLATION AND RT-PCR

BMM cells were treated with RANKL and M-CSF as previously mentioned above. Total RNA was isolated with commercial reagents according to manufacturer's instructions (Qiagen, Victoria, Australia). For RT-PCR, single-stranded cDNA was prepared from 2 μg of total RNA using reverse transcriptase with oligo-dT primer [Ang et al., 2007]. Two microlitres of each cDNA was subjected to PCR amplification using specific primers for cathepsin K, calcitonin receptor, TRAP, DC-STAMP, and V-ATPase d2 [Yip et al., 2006; Feng et al., 2009] and conditions listed in Table I.

TABLE I. Primer Sequences for PCR

Primers	Forward	Reverse	TM (°C)	GenBank No.
Cathepsin K	5′-GGGAGAAAAACCTGAAGC-3′	5′-ATTCTGGGGACTCAGAGC-3′	55	NM_007802.3
Calcitonin receptor	5′-TGGTTGAGGTTGTGCCCA-3′	5′-CTCGTGGGTTTGCCTCATC-3′	62	NM_007588.2
TRAP	5′-TGTGGCCATCTTTATGCT-3′	5′-GTCATTTCTTTGGGGCTT-3′	60	NM_007388.2
V-ATPase d2	5′-ATGCTTGAGACTGCAGAG-3′	5′-TTATAAAATTGGAATGTAGCT-3′	58	NM_175406.2
DC-STAMP	5′-CTTGCAACCTAAGGGCAAAG-3′	5′-TCAACAGCTCTGTCGTGACC-3′	60	NM_029422.2
36B4	5′-TCATTGTGGGAGCAGACA-3′	5′-TCCTCCGACTCTTCCTTT-3′	58	NM_007475.3

NF-κB LUCIFERASE REPORTER GENE ASSAY

To examine NF-κB activation, RAW264.7 cells stably transfected with a luciferase reporter gene p-NF-κB-TA-Luc [Wang et al., 2003], were plated in 24-well plates at a density of 1×10^5 cells/well and pre-treated with mangiferin for 1 h, followed by RANKL (100 ng/ml) stimulation for a further 8 h. Cells were harvested and luciferase activity measured using the Promega Luciferase Assay System according the manufacturer's instructions (Promega).

WESTERN BLOT ANALYSIS OF IκB-α AND PHOSPHORYLATED ERK

Proteins were separated by SDS–PAGE gel and electroblotted onto nitrocellulose membranes (Bio-Rad). Membranes were blocked with 5% (w/v) skim milk powder (SMP) in TBST (10 mM Tris, pH 7.5, 150 mM NaCl, 0.1% (v/v) Tween 20) and then probed with primary antibodies to phosphorylated forms of ERK, IκB-α, and α-tubulin (Santa Cruz Biotechnology, Inc.) in 1% (w/v) SMP in TBST. After washing three times with 1× TBST, membranes were incubated with HRP-conjugated secondary antibodies diluted 1/5,000 in 1% (w/v) SMP in TBST. Membranes were then developed using the ECL system (Amersham Pharmacia Biotech, Sydney, Australia).

IMMUNOHISTOCHEMISTRY OF p65 IN BMM

2×10^4 BMM/well/100 μl were seeded in a 96 plate and incubated at a 37°C incubator overnight. The cells were pre-incubated with mangiferin for 1 h before RANKL (100 ng/ml) was added per well for 30 min. The cells were washed with 1× PBS and fixed with 4% paraformaldehyde for 20 min, followed by three washes with 1× PBS. The cells were permeabalized with 0.1% Triton X-100 in 1× PBS for 5 mins, followed by two washes with 0.1% BSA–PBS. Fifty microlitres of anti-p65 or IgG antibody (Santa Cruz Biotechnology, Inc.) in a dilution of 1:200 was added to each well and the plates were incubated at 37°C for 45 min. The cells were washed four times with 0.1% BSA–PBS, four times with 1× PBS and once with 0.1% BSA–PBS. Streptavidin-horseradish peroxidase (Dako, Victoria, Australia) was added per well and incubated at room temperature for 20 min. After washes, Dako Liquid Dab (Dako) was added for 30 min or until brown color developed.

STATISTICAL ANALYSES

Data presented were representative results from a triplicate set of three independent experiments or the mean ± SEM of those experiments. Student's *t*-test was used to test statistical significance between groups. A *P*-value of <0.05 was considered to be statistically significant.

RESULTS

MANGIFERIN ABROGATES RANKL-INDUCED OSTEOCLASTOGENESIS

To examine the effect of mangiferin on RANKL-induced osteoclastogenesis, BMM cultures were incubated with various concentrations of mangiferin and evaluated for the formation of OCL cells.

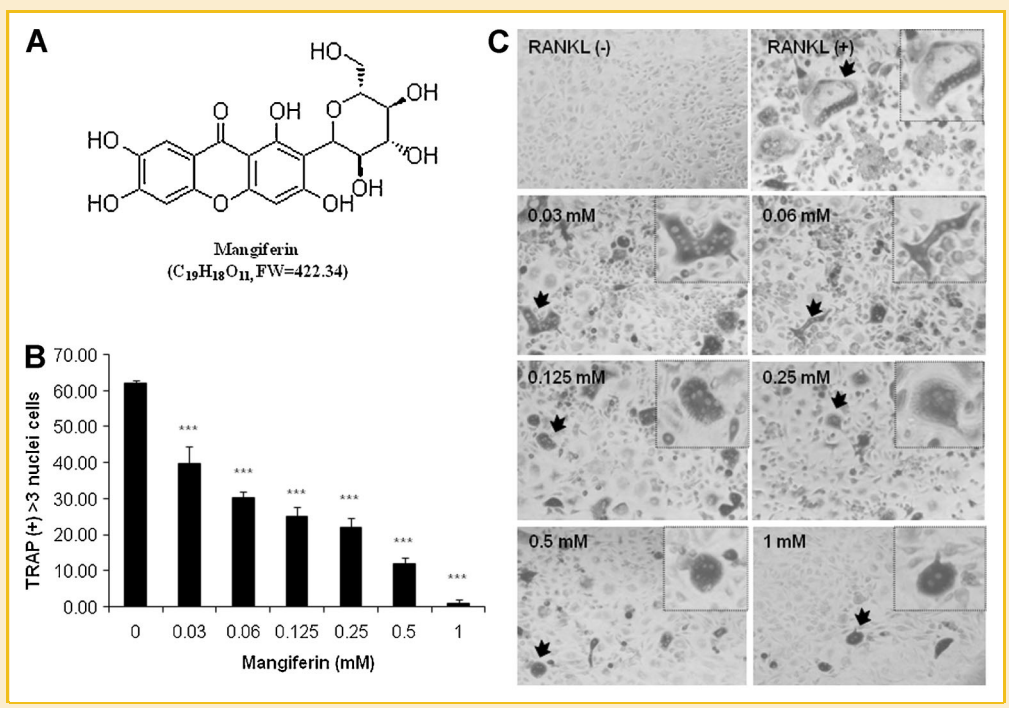

Fig. 1. Mangiferin abrogates RANKL-induced osteoclastogenesis in BMM culture. A: Structure of mangiferin. B: Freshly isolated BMMs were cultured in the presence of M-CSF and RANKL with varying doses of mangiferin. After 7 days of culture, cells were fixed with 4% paraformaldehyde and stained for TRACP activity. Dose-dependent effect of mangiferin on the formation of TRACP (+) OCLs (**P-value < 0.01, ***P-value < 0.001). C: Light microscope images depicting the dose-dependent effect of mangiferin on RANKL-induced osteoclast formation.

TRACP positive cells with more than three nuclei were scored as OCL cells. BMM cultured in the presence of MCSF and RANKL form multinucleated TRACP-positive OCL cells. The addition of mangiferin to BMM cultures showed dose-dependent inhibition of OCL cell formation as measured by the TRACP positive multinucleation cell count (Fig. 1B). Notably, OCL cells cultured in the presence of mangiferin were smaller than that of the control (Fig. 1C). No apparent cell loss was observed at concentrations tested in concentrations up to 500 μM. To further evaluate the morphological changes, we examined the effect of mangiferin on the size of OCL cells during RANKL-induced osteoclast formation using confocal microscopy. The treated cells were double-stained with F-actin and DAPI to allow the visualization of the cytoskeleton and nuclei. In the absence of mangiferin, cells exhibited characteristic osteoclast morphology, including well-spread cytoplasm and numerous nuclei (Fig. 2Aa–c). In contrast, mangiferin-treated OCL cells were smaller in size and had fewer numbers of nuclei (Fig. 2Ad–i,B). Taken together, these data suggest that mangiferin abrogates RANKL-induced osteoclastogenesis.

MANGIFERIN ATTENUATES OSTEOCLASTIC BONE RESORPTION

To test the effect of mangiferin on osteoclastic bone resorption, equal numbers of GCT of bone-derived osteoclasts were seeded onto bone slices and, after attachment, mangiferin was added to cultures. Bone surfaces were retrieved after further 24 h incubation, processed for scanning electron microscopy and the area of resorption pits was measured, as described in Materials and Methods section. Treatment of cultures with mangiferin-attenuated osteoclastic bone resorption (Fig. 3). Note that treatment of mangiferin resulted in shallower resorption pits or reduced pit areas as compared to the untreated control (Fig. 3A,B). Importantly, this decreased bone resorption area did not reflect apoptosis of osteoclastic cells as no significant differences in the total number of TRACP-positive cells per bone

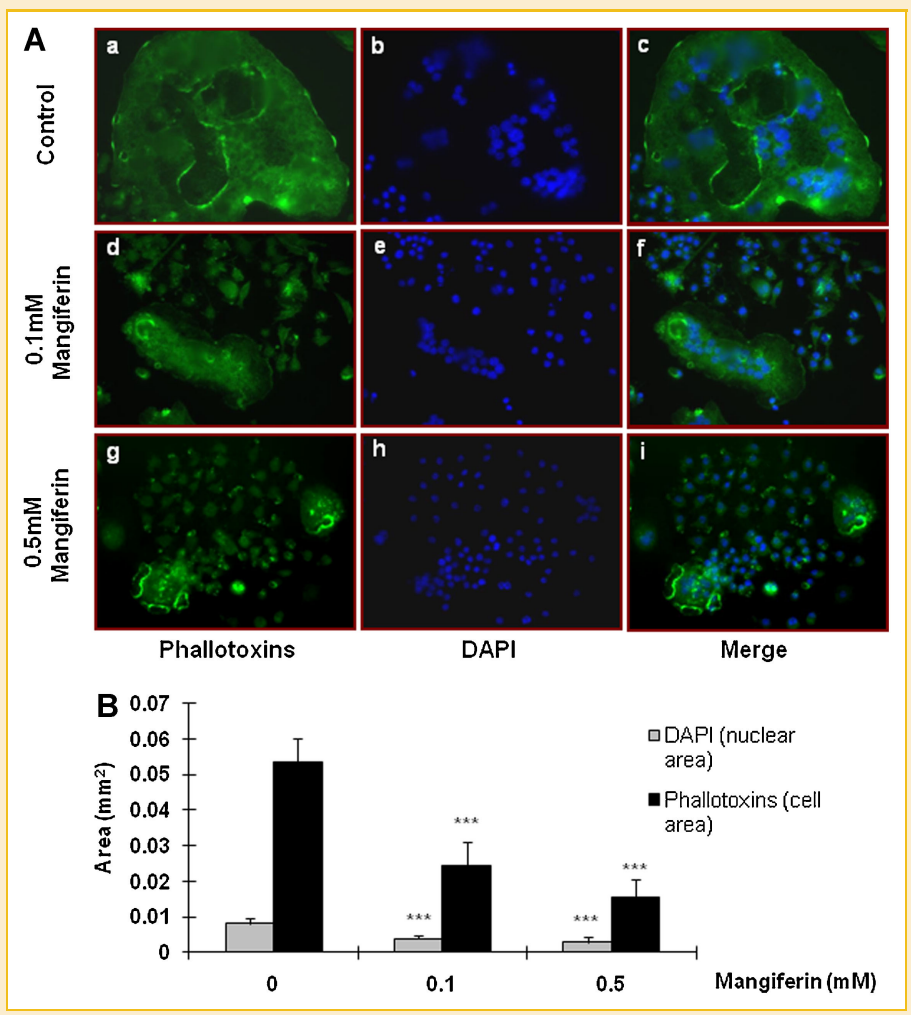

Fig. 2. Mangiferin reduces the size of OCL cells. RAW246.7 cells were cultured in the presence of RANKL with mangiferin in six-well plates seeded with coverslips. After 5 days of culture, cells were fixed with 4% paraformaldehyde and stained stained with DAPI (nuclear staining) and phallotoxins (F-actin structure) antibodies. A: Confocal images of OCL cells left untreated (a–c), treated with 0.1 mM mangiferin (d–f) and 0.5 mM mangiferin (g–i). B: Total cell spread areas and nuclear areas of OCLs from an average of five randomly selected fields (400×) were measured and graphed (***P-value < 0.001).

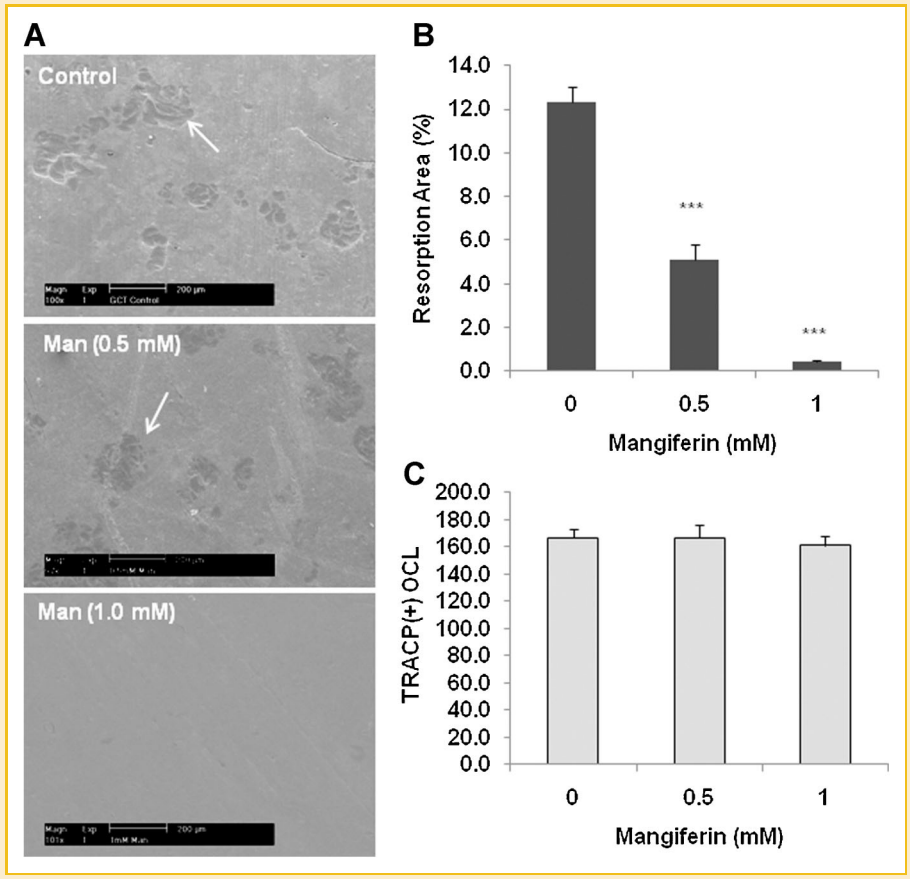

Fig. 3. Inhibition of bone resorption by mangiferin. Equal number of osteoclast-like cells derived from patients presenting with giant cell tumor (GCT) of bone were seeded to bovine bone slices and permitted to attach before the exposing to mangiferin at varying doses (0, 0.5, and 1 mM) for 24 h. A: Representative SEM images of bone resorption pits are shown (arrows). B: The summed areas of resorption pits were measured under SEM and are presented graphically. (***P-value < 0.001). C: Total number of TRACP positive OCL cells per bone slice.

slice were observed after the treatment of mangiferin (Fig. 3C). These experiments indicate that mangiferin inhibited osteoclastic bone resorption.

MANGIFERIN SUPPRESSES RANKL-INDUCED GENE EXPRESSION

To further determine the role of mangiferin in osteoclast differentiation, we examined the effect of mangiferin on the gene expression of cathepsin K, calcitonin receptor and TRAP, dendritic cell-specific transmembrane protein (DC-STAMP) and V-ATPase d2 (d2), all marker genes of osteoclasts. BMM cultures were treated with M-CSF and RANKL in the absence or presence of mangiferin for 5 days followed by total RNA isolation. Semi-quantitative RT-PCR was performed using primers for cathepsin K, calcitonin receptor, TRAP, DC-STAMP, and d2 (Table I). Mangiferin reduced the gene expression of osteoclastic gene markers cathepsin K, calcitonin receptor, and TRAP in a dose-dependent manner during RANKL-induced osteoclastogenesis (Fig. 4), consistent with its inhibitory effects on osteoclastogenesis and bone resorption. In addition, mangiferin also diminished osteoclast fusion gene (DC-STAMP and d2) expression.

MANGIFERIN SUPPRESSES RANKL-INDUCED NF-κB ACTIVATION AND PREVENTS IκB-α DEGRADATION

To measure the inhibitory effect of mangiferin on RANKL-induced NF-κB transcriptional activity, RAW264.7 cells that had been stably transfected with a NF-κB luciferase reporter construct [Wang et al., 2003] were stimulated with RANKL (100 ng/ml) in the presence or absence of mangiferin. In the absence of RANKL, mangiferin increases the basal level of NF-κB luciferase activity. Eight hours post-RANKL treatment, the control showed an increase in the NF-κB luciferase activity. Pre-treatment (1 h) of the cells with mangiferin prior to RANKL stimulation (8 h) dose-dependently reduced NF-κB luciferase activation by more than half (Fig. 5A).

In addition, we tested the effect of mangiferin on RANKL-induced IκB-α degradation. IκB-α degradation was observed at 10 and 20 min post-RANKL-stimulation with maximum degradation observed at 20 min as compared to unstimulated controls. In the presence of mangiferin, IκB-α degradation induced by RANKL was suppressed at both a dose- and time-dependent manner (Fig. 5B). Interestingly, in the absence of RANKL, the basal level of IκB-α was increased by mangiferin at 1 h and 20 min post-treatment.

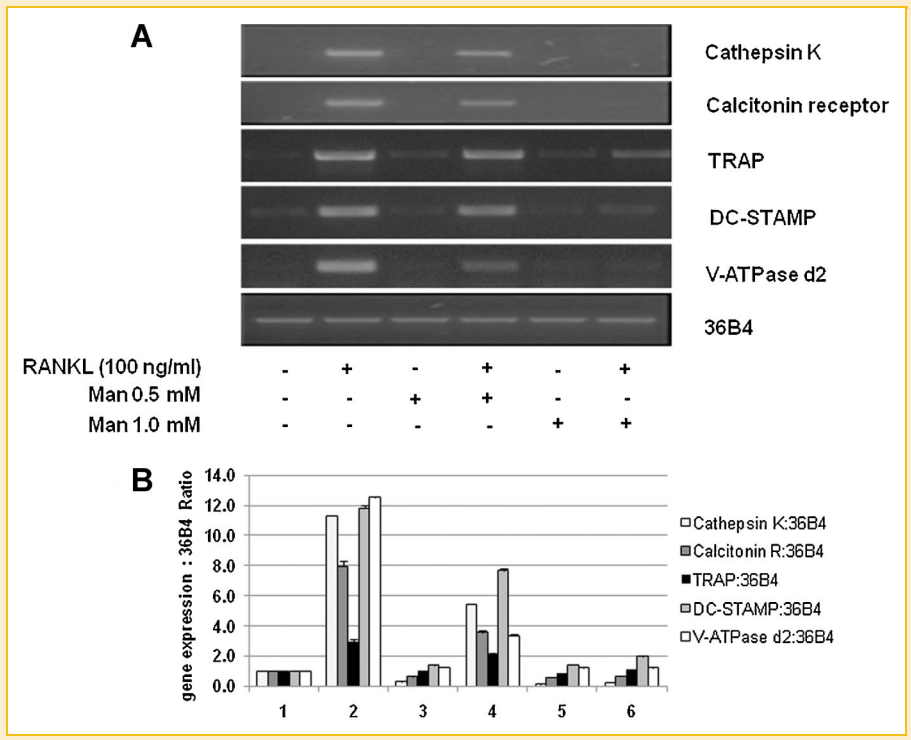

Fig. 4. Mangiferin dose-dependently reduce RANKL-induced gene expression (A,B). BMM cells were seeded onto six-well plates in the presence and absence of RANKL (100 ng/ml) for 5 days with various doses of mangiferin (0, 0.5, and 1 mM). Total cellular RNA was extracted and cDNA was synthesized using 2 μg of total RNA with oligo-dT. PCR amplification was performed using specific primers for cathepsin K, calcitonin receptor, TRAP, DC-STAMP, d2, and 36B4 genes. A: PCR products were separated on 1.2% agarose gels. B: The relative levels of gene expression are shown as the ratios of cathepsin K, calcitonin receptor, TRAP, DC-STAMP, d2 to 36B4 genes.

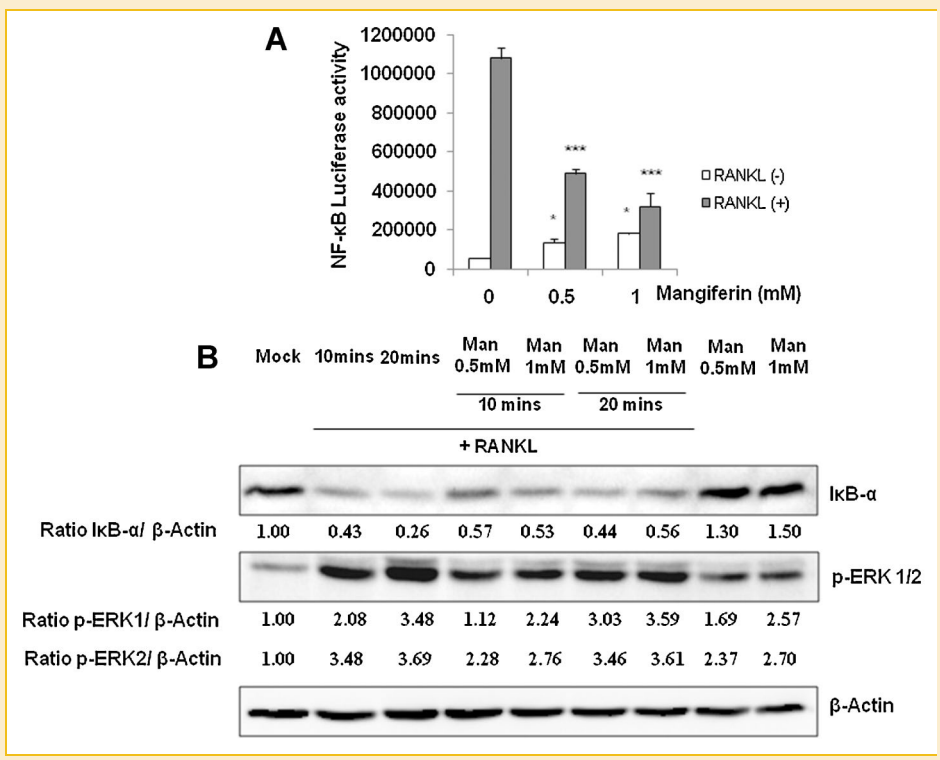

Fig. 5. Mangiferin suppresses RANKL-induced NF-κB activation, prevents IκB-α degradation and inhibits ERK1/2 phosphorylation. A: RAW264.7 cells stably transfected with the 3kB-Luc-SV40 reporter gene were pre-incubated with varying doses of mangiferin for 1 hr followed by RANKL (100 ng/ml) stimulation. Luciferase activity in the lysates was determined after 8 h of RANKL stimulation. Each bar represents the mean ± SE from triplicate wells (**P-value < 0.01, ***P-value < 0.001). B: RAW264.7 cells were pre-treated with mangiferin for 1 h prior to RANKL (100 ng/ml) stimulation for 0, 10, or 20 mins. Proteins from whole cell extracts were separated and transferred to PVDF membranes, which were then blocked and probed sequentially with antibodies to IκB-α, pERK1/2, and β-actin. Bands were visualized by ECL and quantitated by densitometry. Results from a representative of triplicate experiments are shown. The levels of IκB-α, pERK1/2 proteins are shown as a ratio to β-actin.

MANGIFERIN SUPPRESSES RANKL-INDUCED ERK PHOSPHORYLATION

To further explore pathways by which mangiferin regulates osteoclast differentiation and function, RANKL-induced ERK phosphorylation was examined. To this end, RAW264.7 cells were exposed to mangiferin in the presence and absence of RANKL. Western blot analyses demonstrated significant ERK phosphorylation after 10 and 20 min of RANKL treatment alone. However, in the presence of mangiferin, an inhibitory effect on RANKL-induced ERK phosphorylation was observed (Fig. 5B). In comparison, in the absence of RANKL, the basal level of ERK1/2 was increased in the presence of mangiferin.

MANGIFERIN INHIBITS p65 NUCLEAR TRANSLOCATION

Next, we examined the effect of mangiferin on p65 nuclear translocation. BMMs were pretreated with mangiferin for 1 h and stimulated with RANKL (100 ng/ml). As shown in Figure 6, mangiferin decreased the degree of p65 nuclear translocation induced by RANKL, consistent with its inhibitory effect on NF-κB luciferase activity and IκB-α degradation.

DISCUSSION

In this study, we revealed for the first time that mangiferin inhibits osteoclast formation and bone resorption via the suppression of RANKL-induced activation of NF-κB and ERK. Interestingly, mangiferin is one of the major ingredients used in traditional Chinese medicine formula, Er-Xian decoction, which has long been used for the treatment of post-menopausal osteoporosis in China. Mangiferin was found to exhibit anti-osteoporotic effects in ovariectomized rats [Qin et al., 2008]. Thus, dissecting the mechanism of action of this natural compound at both cellular and molecular level might provide invaluable information for its use as a potential treatment for osteoporosis.

During RANKL-induced osteoclastogenesis, RANKL binds to its receptor RANK resulting in the recruitment of adaptor protein, TRAF6 and the subsequent activation of downstream NF-κB and MEK kinases [Hsu et al., 1999; Zhang et al., 2001; Yamamoto et al., 2002]. NF-κB signaling has been shown to play an indispensable role in osteoclastogenesis [Boyce et al., 1999]. NF-κB $p50^{-/-}$ and $p52^{-/-}$ double knockout mice exhibit severe osteopetrosis due to the

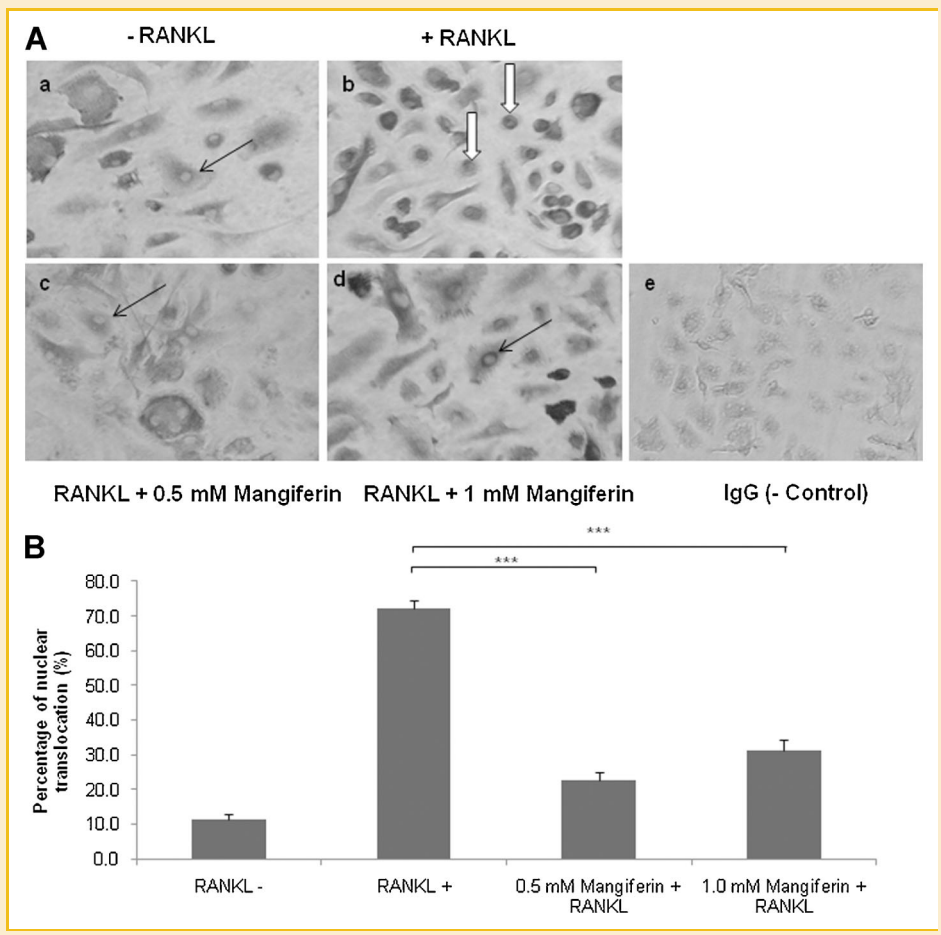

Fig. 6. Mangiferin inhibits RANKL-induced p65 nuclear translocation. BMM cells were pre-incubated with mangiferin for 1 h before the addition of RANKL (100 ng/ml) for 30 mins. Cells were fixed with 4% paraformaldehyde and stained with anti-p65 antibody. A: Note that RANKL-induced nuclear translocation of p65 (comparing a to b) was inhibited by pretreatment with mangiferin at both concentrations (c and d). IgG antibody staining was used as a negative control (e). Black arrows (a, c, and d) depict localization of p65 in the cytoplasm and white arrows (b) indicate presence of p65 in the nucleus. (B) The percentage of cells showing nuclear translocation was calculated. Each bar represents the mean ± SE from triplicate wells (***P-value < 0.001).

failure of osteoclast formation [Franzoso et al., 1997; Iacovino, 2001]. Therefore, the suppression of NF-κB activation would play an important role in the inhibition of osteoclast formation and osteolytic bone conditions [Xu et al., 2009]. Interestingly, mangiferin was found to reduce RANKL-induced NF-κB-activity, resulting in the inhibition of RANKL-induced osteoclast differentiation. In line with the findings from this study, mangiferin has been shown to inhibit TNF-induced NF-κB activation and reactive oxygen intermediate (ROI) generation, indicating that mangiferin might serve as a potent drug for anti-inflammatory and antioxidant therapy [Sarkar et al., 2004]. These anti-NF-κB effects of mangiferin could further explain the underlying mechanism of action in the inhibition of osteoclastogenesis and bone resorption. Furthermore, we have shown that mangiferin prevented the RANKL-induced degradation of IκB-α and the nuclear translocation of p65. Intriguingly, in the absence of RANKL stimulation, mangiferin appeared to increase the basal level of NF-κB activity (Fig. 5A). However, magiferin also appeared to increase the IκB-α levels, 1 h and 20 min post-treatment (Fig. 5B), which might reflect the resynthesis of IκB-α following its degradation. The MEK/ERK pathway is also involved in the regulation of osteoclast differentiation [Miyazaki et al., 2000; Hotokezaka et al., 2002]. We have demonstrated that mangiferin inhibits RANKL-induced activation of ERK, which might further contribute to the suppression of osteoclast differentiation. The effect of mangiferin on MEK/ERK has not been well documented in other cell types; hence, further investigations are essential to understand the effect of mangiferin on these signaling pathways. Collectively, our data suggest that mangiferin attenuates osteoclast formation and function through the suppression of NF-κB and ERK pathways.

A previous study has shown that mangiferin display a free radical scavenging ability against H_2O_2-induced damage of red blood cells [Rodriguez et al., 2006]. It also inhibits Fe^{2+}-citrate-induced lipoperoxidation in isolated rat liver mitochondria [Pardo Andreu et al., 2005]. In addition, mangiferin is able to inhibit the expression of iNOS and TNF-alpha genes that regulate macrophage and neuron cells [Leiro et al., 2003, 2004]. Furthermore, mangiferin is able to attenuate PGE2 production; free radical formation and COX-2 synthesis induced by LPS, and thus might have a protective effect for cerebral microglial [Bhatia et al., 2008]. Whether mangiferin exhibits similar effects in OCL cells remain to be elucidated.

In summary, our findings clearly demonstrate that mangiferin inhibits osteoclast formation and function via the suppression of RANKL-induced IκB-α degradation and nuclear translocation of p65, which in turn decreases RANKL-induced NF-κB activation. Mangiferin also inhibited RANKL-induced ERK phosphorylation. Our results suggest potential therapeutic benefits of mangiferin for the prevention and treatment of osteolytic bone diseases.

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