Biochemical and Biophysical Research Communications 430 (2013) 951–956

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

# Sanguinarine inhibits osteoclast formation and bone resorption via suppressing RANKL-induced activation of NF-κB and ERK signaling pathways

Haowei Li [a,1], Zanjing Zhai [a,1], Guangwang Liu [a,b], Tingting Tang [a], Zhen Lin [c,d], Minghao Zheng [d], An Qin [a,d,*], Kerong Dai [a,*]

[a] Shanghai Key Laboratory of Orthopaedic Implants, Department of Orthopaedics, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, PR China
[b] Department of Orthopaedic Surgery, The Central Hospital of Xuzhou, Affiliated Hospital of Medical Collage of Southeast University, PR China
[c] Division of Orthopaedic, Department of Surgery, Guangdong Academy of Medical Sciences, Guangdong General Hospital, Guangdong, PR China
[d] Centre for Orthopaedic Research, School of Surgery, The University of Western Australia, Perth, Australia

ARTICLE INFO

*Article history:*
Received 15 November 2012
Available online 19 December 2012

*Keywords:*
Sanguinarine
Osteoclast
Bone resorption
NF-κB
ERK

ABSTRACT

Sanguinarine is a natural plant extract that has been supplemented in a number of gingival health products to suppress the growth of dental plaque. However, whether sanguinarine has any effect on teeth and alveolar bone health is still unclear. In this study, we demonstrated for the first time that sanguinarine could suppress osteoclastic bone resorption and osteoclast formation in a dose-dependent manner. Sanguinarine diminished the expression of osteoclast marker genes, including TRAP, cathepsin K, calcitonin receptor, DC-STAMP, V-ATPase d2, NFATc1 and c-fos. Further investigation revealed that sanguinarine attenuated RANKL-mediated IκBα phosphorylation and degradation, leading to the impairment of NF-κB signaling pathway during osteoclast differentiation. In addition, sanguinarine also affected the ERK signaling pathway by inhibiting RANKL-induced ERK phosphorylation. Collectively, this study suggested that sanguinarine has protective effects on teeth and alveolar bone health.



## 1. Introduction

Multi-nucleated osteoclast that arises from the hematopoietic stem cell monocyte/macrophage lineage is the principal contributor to teeth eruption and root bone resorption [1]. Excessive osteoclast formation and bone resorption are closely related with diseases such as osteoporosis, periodontitis and tooth loss [2,3]. Current molecular findings have demonstrated that the receptor activator of nuclear factor-κB ligand (RANKL) plays a key role in osteoclast differentiation and activation [4–6]. The binding of RANKL to its receptor RANK leads to a cascade of intracellular events including: (1) recruitment of TRAFs; (2) activation of the transcription factor nuclear factor-κB (NF-κB); and (3) activation of the mitogen-activated protein kinases (MAPKs) pathways including extracellular signal-regulated kinase 1/2 (ERK1/2), p38 and c-Jun *N*-terminal kinase (JNK) [5,6]. Targeted blockage of these signaling pathways might suppress osteoclast formation/function, and thus have therapeutic value in treating osteoclast-related diseases.

Sanguinarine is the active ingredient derived from the roots of Sanguinaria Canadensis [7]. It is a FDA approved compound that has been commonly used as a supplement for several gingival health products to suppress the growth of dental plaque [8]. Several studies have demonstrated sanguinarine exhibited anti-inflammatory, anti-tumor, anti-microbial, and anti-oxidant properties [9–12]. Although it has been widely used, the effect of sanguinarine on bone biology, such as the alveolar bone, is still unclear. Therefore, this study aims to investigate whether the supplement sanguinarine in gingival health products has any effect on bone cells.

## 2. Materials and methods

### 2.1. Media and reagents

RAW264.7 cells were obtained from the American Type Culture Collection (Rockville, MD). Alpha modification of eagles medium (α-MEM) and fetal bovine serum (FBS) were purchased from Gibco-BRL (Sydney, Australia). Sanguinarine was purchased from Herbest (Baoji, Shannxi China) and was dissolved in Dimethyl

* Corresponding authors. Address: Shanghai Key Laboratory of Orthopaedic Implants, Department of Orthopaedics, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhizaoju Road, Shanghai 200011, P.R.China; Faculty of Medicine, Dentistry and Health Sciences, The University of Western Australia (M508), 35 Stirling Highway, CRAWLEY WA 6009, Australia. Fax: +86 21 63139920, +61 8 93464050 (A. Qin), Shanghai Key Laboratory of Orthopaedic Implants, Department of Orthopaedics, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhizaoju Road, Shanghai 200011, P.R.China. Fax: +86 21 63139920 (K.Dai).

*E-mail addresses:* dr.qinan@gmail.com (A. Qin), krdai@163.com (K. Dai).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.12.051

sulfoxide (DMSO). The Prime Script RT reagent Kit and SYBR® Premix Ex Taq™ II was obtained from TaKaRa Biotechnology (Otsu, Shiga, Japan). The luciferase assay system was obtained from Promega (Sydney, Australia). Soluble human recombinant macrophage-colony stimulating factor (M-CSF) and bacteria-derived recombinant mouse RANKL were supplied by R&D (R&D Systems, Minneapolis, MN). Specific antibodies against β-actin, phospho-ERK1/2, ERK1/2, phospho-IκBα and IκBα were purchased from Cell Signaling (Danvers, MA). The Diagnostic Acid Phosphatase kit for tartrate-resistant acid phosphatase (TRAP) staining solution were obtained from Sigma Chemical, the reagent of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Tris, glycine, NaCl, SDS, and other regents were obtained from Sigma (St. Louis, MO, USA).

### 2.2. Cell culture

Primary mouse bone marrow monocyte/macrophage (BMM) cells were isolated from the long bones of 5-week-old C57BL/6 mice and cultured in complete α-MEM medium (α-MEM containing 10% heat inactivated FBS, 2 mM L-glutamine, 100 U/ml penicillin/streptomycin and 30 ng/ml M-CSF). RAW264.7 cells were cultured in α-MEM containing 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin/streptomycin. The cell cultures were maintained at 37 °C in a humid environment with 5% $CO_2$.

### 2.3. Bone resorption assay

BMM cells ($2.4 \times 10^4$ cells/cm$^2$) were seeded onto bovine bone slices with complete α-MEM medium. The BMM cells were stimulated with RANKL (100 ng/ml), M-CSF (30 ng/ml) and sanguinarine (0, 2.5 or 5 μM) until the formation of osteoclast-like (OCL) cells. Then, the OCL cells were removed from bone slices by mechanical agitation and sonication. The resorption pits were visualized under a scanning electron microscope (SEM, FEI Quanta 250). The percentage of resorbed bone surface area was quantified using the Image J software (National Institutes of Health).

### 2.4. In vitro osteoclastogenesis assay

The BMM cells were seeded onto a 96-well plate at a density of $8 \times 10^3$ cells/well supplied with complete α-MEM medium, RANKL (100 ng/ml) and sanguinarine at varying concentrations (0, 2.5, 5 or 10 μM). The culture medium was replaced every 2 days until the formation of osteoclasts at day 7. The cells were fixed with 4% paraformaldehyde for 20 min and stained for TRAP using the Diagnostic Acid Phosphatase kit. TRAP positive cells with more than 3 nuclei were counted as OCL cells.

### 2.5. Cytotoxicity assays

The effect of sanguinarine on the viability of BMM cells was determined by MTT assay. The BMM cells were plated in the 96-well plates at the density of $1 \times 10^5$ cells/well, and cultured in complete α-MEM medium and different doses of sanguinarine (0, 2.5, 5, 10, 20 or 40 μM) for 48 h. MTT assay was then performed according to the manufacturer's instruction. The half-maximal inhibitory concentration ($IC_{50}$) value was calculated by GraphPad Prism program version 5.0c (San Diego, CA, USA).

### 2.6. RNA extraction and quantitative real-time PCR

Quantitative real-time PCR was used to examine the effect of sanguinarine on the gene expression profiles during osteclastogenesis. RANKL induced osteclastogenesis from mouse BMM cells was administrated with either different doses of sanguinarine (0, 2.5 or 5 μM) for 5 days or 5 μM sanguinarine for 0–4 days. Total RNA was extracted using the Qiagen Rneasy Mini kit (Qiagen, Victoria, Australia). Single-stranded cDNA was synthesized from 2 μg of total RNA using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Subsequently, real-time PCR was performed on an ABI 7500 Sequencing Detection System using the SYBR® Premix Ex Taq™ II. Briefly, 12.5 μl of SYBR® Premix Ex Taq™ II, 8.5 μl $ddH_2O$, 2 μl cDNA and 1 μl of each primer were mixed to make up a total volume of 25 μl for each PCR. Cycling condition was 95 °C 5 s and 60 °C 34 s for 40 cycles with specific primers for cathepsin K (CTSK), calcitonin receptor (CTR), TRAP, dendritic cell-specific transmembrane protein (DC-STAMP), V-ATPase d2, c-fos and NFATc1 [13–17]. Beta-actin was included as housekeeping gene. The primer sequences were listed in Suppl. Table I. The comparative $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression levels of each gene as previously described [18].

### 2.7. NF-κB luciferase reporter gene assays

To investigate whether sanguinarine could affect the NF-κB signaling pathway during osteoclast differentiation, the RAW264.7 cells that had been stably transfected with a NF-κB luciferase reporter construct (p-NF-κB-TA-Luc) were used to determine the NF-κB activation as previously described [19]. Briefly, the cells were plated on a 24-well plate at a density of $1 \times 10^5$ cells/well and pretreated with sanguinarine (0–20 μM) for 1 h, followed by RANKL stimulation for a further 8 h before cell harvest. The luciferase activity was measured using the Promega Luciferase Assay System.

### 2.8. Western blot analysis

After pretreatment of vehicle or sanguinarine for 2 h, the RAW264.7 cells were stimulated with RANKL for 0, 5, 10, 20, 30 or 60 min. The cells were then washed in 1× PBS twice and lysed in ice-cold lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 1% sodium deoxycholate) supplemented with PMSF (Shen Neng Bo Cai Corp., China). The lysates were incubated on ice for 30 min and centrifuged at 12,000 rpm for 10 min to precipitate the debris. The protein concentrations were determined using the BCA protein assay kit (Thermo Scientific, Rockford). Equal amount of the protein lysates were separated using a 10% SDS–PAGE and electroblotted onto PVDF membranes (Roche). The membranes were then blocked with 5% (wt/vol) skim milk solution for 1 h and probed with the primary antibodies (β-actin, 1:1000; phospho-ERK1/2, 1:1000; ERK1/2, 1:1000; phospho-IκBα, 1:1000; and IκBα, 1:1000) at room temperature for 4 h. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies (1:5000) for 1 h. After that, the antibody reactivity was visualized by exposure in an Odyssey infrared imaging system (Li-COR).

### 2.9. Statistical analyses

Statistically significant differences between groups were determined by Student's *t*-test using the SPSS 13.0 software (SPSS Inc., USA). *P* values less than 0.05 were considered statistically significant.

## 3. Results

### 3.1. Sanguinarine attenuates osteoclastic bone resorption

Firstly, we examined the direct effect of sanguinarine on osteoclastic bone resorption in vitro. As revealed by SEM, osteoclasts without sanguinarine treatment actively resorbed the bone surface. In contrast, more than 50% of the bone resorption activity was effectively inhibited by sanguinarine at 2.5 μM, with almost

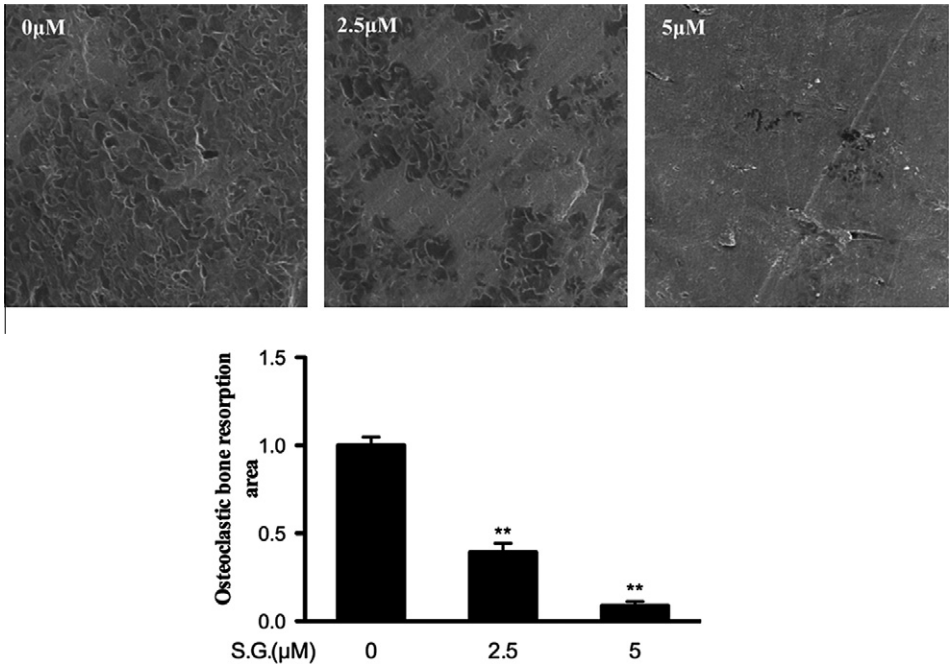


**Fig. 1.** Sanguinarine (S.G.) attenuates osteoclastic bone resorption in vitro. Equal number of BMM cells were seeded onto bovine bone slices and allowed to adhere to the surface before the addition of M-CSF (30 ng/ml), RANKL (100 ng/ml) and sanguinarine at varying doses (0, 2.5 and 5 μM) for 7 days. (A) Representative S.E.M. images of bone resorption pits are shown. (B) The total areas of resorption pits were measured using image J, and are presented graphically. Similar results were obtained from at least 3 independent experiments (**$p$<0.01).

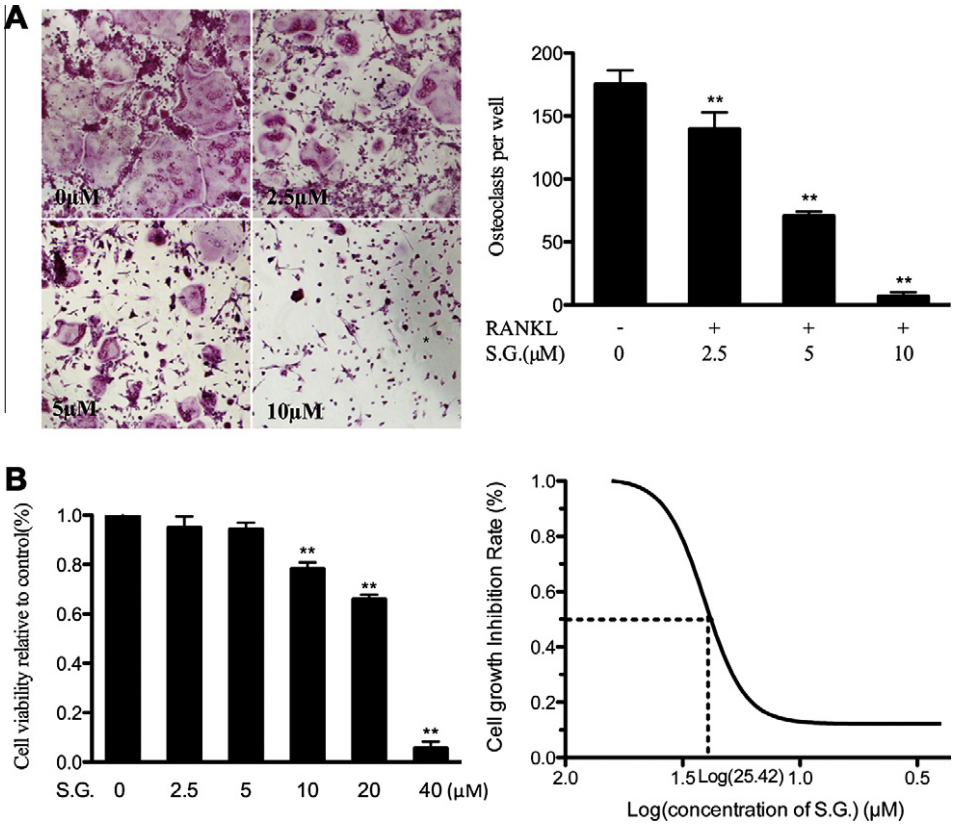


**Fig. 2.** Sanguinarine inhibits RANKL-induced osteoclast formation. (A) BMM cells were cultured in complete α-MEM medium supplemented with RANKL (100 ng/ml) and the indicated concentrations of sanguinarine. After 7 days, the cells were fixed with 4% paraformaldehyde and subjected to TRAP staining (left). The number of TRAP-positive, multinucleated (>3 nucleus) osteoclasts was counted as osteoclast-like (OCL) cells (right). (B) BMM cells were cultured in complete α-MEM medium and the indicated concentrations of sanguinarine for 48 h. The cell viability relative to control was measured using MTT reagent (left). The Inhibition Rate of BMM cells was calculated using GraphPad Prism software (right), and the half-maximal inhibitory concentration ($IC_{50}$) was 25.42 μM (*$p$<0.05; **$p$<0.01).

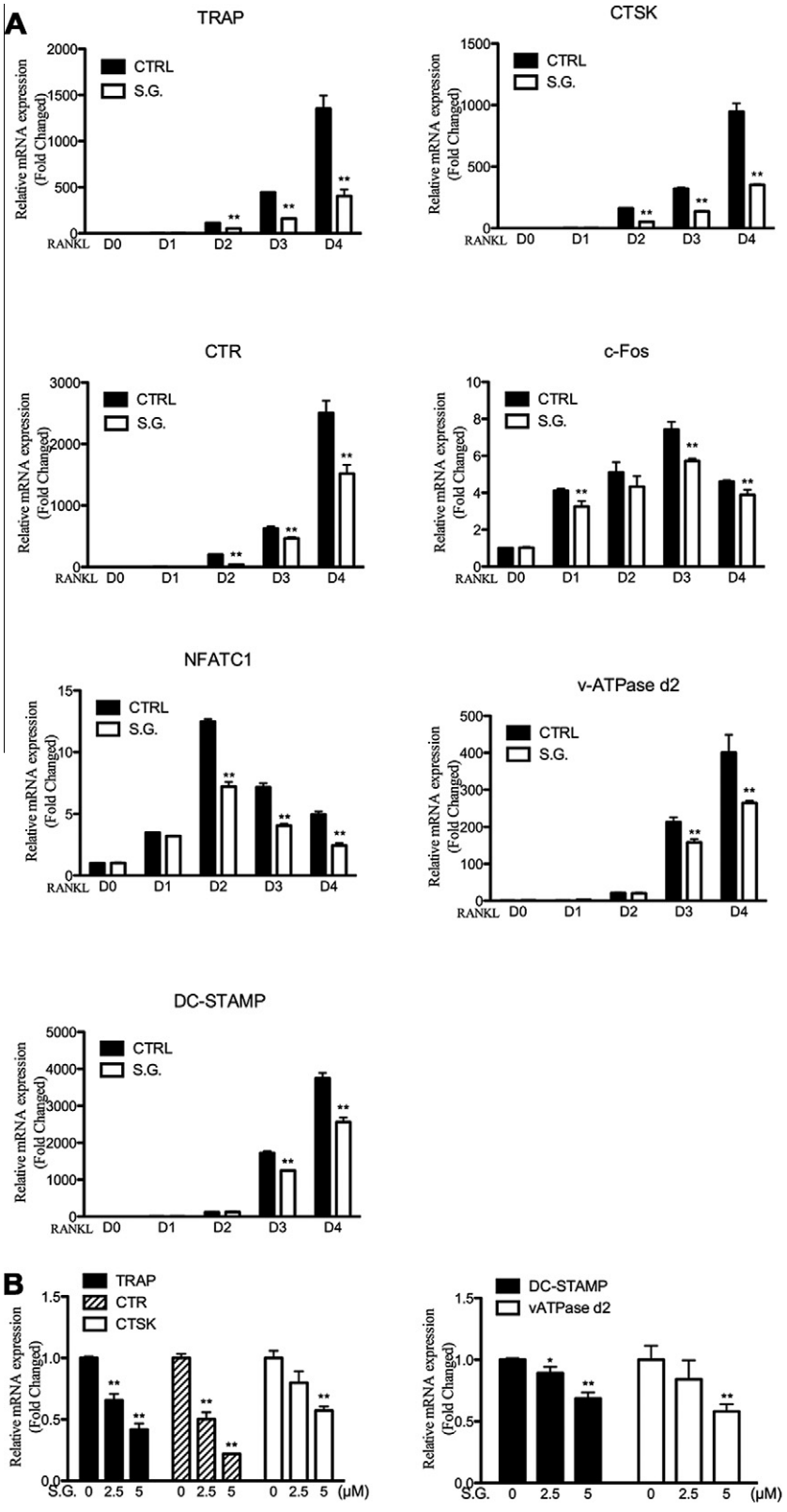


**Fig. 3.** Sanguinarine suppresses RANKL-induced gene expression. BMM cells were cultured with M-CSF (30 ng/ml), RANKL (100 ng/ml) and the indicated concentrations of sanguinarine for 5 days or with M-CSF (30 ng/ml), RANKL (100 ng/ml) and 5 μM sanguinarine for 0, 1, 2, 3 or 4 days. RANKL-inducible gene expression was analyzed by real-time PCR. RNA levels were normalized relative to the expression of β-actin (*$p$<0.05; **$p$<0.01).

completely blockade of bone resorption attained at higher concentrations (>5 μM) (Fig. 1). This result clearly demonstrated that the administration of sanguinarine reduced bone resorption in vitro.

### 3.2. Sanguinarine inhibits RANKL-induced osteoclast formation

To assess whether the effect of sanguinarine on osteoclastic bone resorption is due to the inhibition of RANKL-induced

osteoclast differentiation, osteoclastogenesis was assessed by TRAP staining. In the control group, the BMM cells differentiated into characteristic TRAP positive multinucleated OCL cells (Fig. 2A). However, in the sanguinarine treated groups, the number of TRAP positive OCL cells was significantly decreased in a dose dependent manner. Approximately 50% osteoclast formation was suppressed by sanguinarine at 5 μM (Fig. 2A).

To exclude the possibility that the decrease in OCL cell number was due to the cytotoxicity effect of sanguinarine, MTT assay was preformed. The $IC_{50}$ value of sanguinarine was at 25.42 μM. We found that sanguinarine might partially suppress the proliferation of BMM cells only at high concentrations (>10 μM). However, sanguinarine at either 2.5 or 5 μM did not affect the proliferation of BMM cells (Fig. 2B). The result suggested that sanguinarine at lower dose could impair osteoclast formation without causing cell death.

### 3.3. Sanguinarine suppresses RANKL-induced gene expression

To further elucidate the role of sanguinarine on osteoclast differentiation, we examined its effect on the expression of the osteoclastic marker genes during osteoclastogenesis. As shown, the osteoclastic markers, including TRAP, CTSK, CTR, DC-STAMP and V-ATPase d2, were significantly upregulated upon RANKL treatment at day 7. The expression levels of transcription factors c-fos and NFATc1 reached their peaks at the mid-phrase of osteoclast differentiation (Fig. 3A). However, the up-regulation of osteoclastic marker genes (TRAP, CTSK, CTR, DC-STAMP and V-ATPase d2) was attenuated by the addition of sanguinarine (5 μM, Fig. 3A). Upregulation of c-fos and NFATc1 expression was also diminished by sanguinarine treatment. Consistent with the inhibitory effect on osteoclast formation, sanguinarine also suppressed the upregulation of these osteoclastic marker genes in a dose dependent (0, 2.5 and 5 μM) manner (Fig. 3B). Taken together, sanguinarine altered the gene expression profile during osteoclast differentiation.

### 3.4. Sanguinarine suppresses RANKL-induced activation of NF-κB and ERK signaling pathways

In order to elucidate the underlying mechanism of the inhibitory effect of sanguinarine on osteoclast formation and function, we examined whether sanguinarine could suppress RANKL-induced NF-κB activation. As shown in Fig. 4A, NF-κB was activated by RANKL. In contrast, the activation of NF-κB was partially reduced by sanguinarine in a dose dependent manner. The results suggested that sanguinarine might participate in the regulation of the RANKL-activated NF-κB signaling pathway, and thus contribute to the inhibition of osteoclast formation (Fig. 4A). This finding was further supported by Western blot analysis of IκBα phosphorylation and degradation. In the control cells, IκBα phosphorylation and degradation were initiated by RANKL stimulation (Fig. 4B, left). When the cells were pretreated with sanguinarine (5 μM), the RANKL-induced IκBα phosphorylation and degradation were both significantly suppressed (Fig. 4B, right). These data firmly suggested that sanguinarine suppressed RANKL-induced NF-κB signaling pathway by inhibiting IκBα phosphorylation and degradation.

To further explore the signaling pathways mediated by sanguinarine during osteoclastogenesis, we examined the effect of sanguinarine on RANKL-induced ERK1/2 phosphorylation. As shown, the phosphorylation of ERK1/2 reached its peak within 20 min of RANKL stimulation (Fig. 4B, left). However, pretreatment with sanguinarine significantly decreased the phosphorylation of ERK1/2 (Fig. 4B, right). Collectively, these data suggested that sanguinarine also inhibited ERK1/2 signaling pathway during osteoclastogenesis.

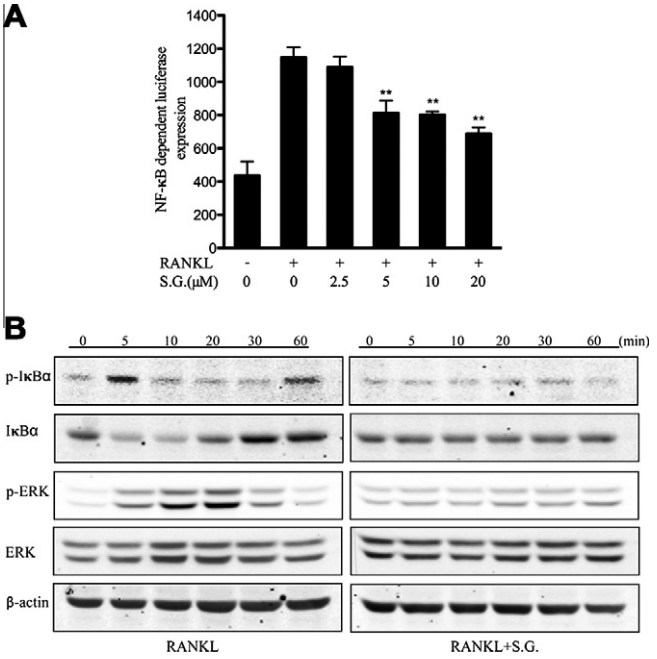


**Fig. 4.** Sanguinarine inhibits RANKL induced NF-κB activation and ERK1/2 phosphorylation. (A) RAW264.7 cells that had been stably transfected with a NF-κB luciferase reporter construct were pretreated with the indicated concentrations of sanguinarine for 1 h and then incubated in the absence or presence of RANKL for 8 h. The luciferase activity was determined by using the Promega Luciferase Assay System. (B) RAW264.7 cells were pretreated with vehicle or sanguinarine for 2 h followed by RANKL stimulation (100 ng/ml) for the indicated times. Then cells were lysed in lysis buffer, and lysates were analyzed by Western blotting with the indicated antibodies (**$p<0.01$).

## 4. Discussion

Sanguinarine is recognized as a quaternary ammonium salt from the family of benzylisoquinoline alkaloids. It is an extract from a number of plants, such as Sanguinaria Canadensis (bloodroot) and Mexican prickly poppy Argemone Mexicana [20]. This natural extract was firstly identified as a toxin that could be used to kill animal cells via its regulation on the $Na^+$–$K^+$-ATPase transmembrane protein [21]. A number of in vitro and in vivo studies have demonstrated that sanguinarine could cause targeted apoptosis in human cancer cells and thus might be used for cancer treatment [22–24]. Other studies suggested that sanguinarine has an anti-proliferative and apoptotic response for cancer cells versus normal cells [25]. Particularly, sanguinarine was widely used as a supplement in a number of gingival health products to suppress the growth of dental plaque, with its effect on alveolar bone biology remaining unknown [8]. In this study, we have verified for the first time that sanguinarine inhibited osteoclast differentiation and bone resorption, suggesting an additional protective effect of sanguinarine on teeth and alveolar bone health.

In addition, we revealed the molecular mechanisms of sanguinarine on osteoclasts are through suppressing RANKL-induced NF-κB and ERK signaling pathways. The RANKL-induced signaling pathways, including NF-κB, ERK, JNK and p38, are essential for osteoclast differentiation and survival [26,27]. NF-κB is a nuclear transcription factor that is ubiquitously present in all cell types. In the resting cells, NF-κB proteins are in the cytoplasm in association with a number of inhibitory IκB proteins including IκBα, IκBβ, and IκBε [28], among which IκBα is the most abundant. NF-κB signaling has been shown to play an indispensable role in osteoclastogenesis [29]. NF-κB $p50^{-/-}$ and $p52^{-/-}$ knockout mice show serious osteopetrosis because of the failure of osteoclast

formation [30,31]. Therefore, the inhibition of NF-κB activation would play a key role in the inhibition of osteoclast formation and osteolytic bone conditions [27]. In this study, we demonstrated that sanguinarine reduced the RANKL-induced NF-κB activity by preventing the IκBα phosphorylation and degradation (Fig. 4), contributing to the inhibition of RANKL-induced osteoclast differentiation.

Interestingly, the levels of ERK1/2 phosphorylation were found to be reduced in the sanguinarine treated cells, suggesting that sanguinarine might disrupt osteoclast differentiation through multiple targets. Further investigations are important to identify the direct target of sanguinarine on this pathway.

In summary, sanguinarine supplemented in gingival health products is capable of inhibiting osteoclast formation and function, indicating additional therapeutic benefits of sanguinarine for oral health. In addition, this study also clearly revealed the molecular mechanisms of sanguinarine on osteoclasts are via impairing NF-κB and ERK signaling pathways in vitro. However, further clinical investigation of sanguinarine on oral health is still required.

## Acknowledgments

This work was supported by Program for Innovative Research Team of Shanghai Municipal Education Commission (Phase I), Grant awarded for Innovative Research from Shanghai Municipal Education Commission (13YZ031), Grant awarded by the Key Disciplines of Shanghai Municipal Education Commission of China (J50206), Grant awarded by NHMRC(Australia) to Prof. Minghao Zheng, and Grant for scientific research from the National Natural Science Foundation for the Youth of China (No. 81201364).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.12.051.

## References

[1] N. Udagawa, N. Takahashi, T. Akatsu, H. Tanaka, T. Sasaki, T. Nishihara, T. Koga, T.J. Martin, T. Suda, Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells, Proc. Natl. Acad. Sci. USA 87 (1990) 7260–7264.
[2] S.H. Wiebe, M. Hafezi, H.S. Sandhu, S.M. Sims, S.J. Dixon, Osteoclast activation in inflammatory periodontal diseases, Oral Dis. 2 (1996) 167–180.
[3] M.H. Helfrich, Osteoclast diseases and dental abnormalities, Arch. Oral Biol. 50 (2005) 115–122.
[4] T. Nakashima, M. Hayashi, T. Fukunaga, K. Kurata, M. Oh-Hora, J.Q. Feng, L.F. Bonewald, T. Kodama, A. Wutz, E.F. Wagner, J.M. Penninger, H. Takayanagi, Evidence for osteocyte regulation of bone homeostasis through RANKL expression, Nat. Med. 17 (2011) 1231–1234.
[5] W.J. Boyle, W.S. Simonet, D.L. Lacey, Osteoclast differentiation and activation, Nature 423 (2003) 337–342.
[6] X. Feng, RANKing intracellular signaling in osteoclasts, IUBMB Life 57 (2005) 389–395.
[7] W.C. Evans, Pharmacognosy, 15th ed., Harcourt Publishers, London, 2002.
[8] T.H. Grenby, The use of sanguinarine in mouthwashes and toothpaste compared with some other antimicrobial agents, Br. Dent. J. 178 (1995) 254–258.
[9] F. Pica, E. Balestrieri, A. Serafino, R. Sorrentino, R. Gaziano, G. Moroni, N. Moroni, G. Palmieri, M. Mattei, E. Garaci, P. Sinibaldi-Vallebona, Antitumor effects of the benzophenanthridine alkaloid sanguinarine in a rat syngeneic model of colorectal cancer, Anticancer Drugs 23 (2012) 32–42.
[10] J. Lenfeld, M. Kroutil, E. Marsalek, J. Slavik, V. Preininger, V. Simanek, Antiinflammatory activity of quaternary benzophenanthridine alkaloids from Chelidonium majus, Planta Med. 43 (1981) 161–165.
[11] L.A. Mitscher, Y.H. Park, D. Clark, G.W. Clark 3rd, P.D. Hammesfahr, W.N. Wu, J.L. Beal, Antimicrobial agents from higher plants. An investigation of Hunnemannia fumariaefolia pseudoalcoholates of sanguinarine and chelerythrine, Lloydia 41 (1978) 145–150.
[12] M.M. Chaturvedi, A. Kumar, B.G. Darnay, G.B. Chainy, S. Agarwal, B.B. Aggarwal, Sanguinarine (pseudochelerythrine) is a potent inhibitor of NF-kappaB activation. IkappaBalpha phosphorylation and degradation, J. Biol. Chem. 272 (1997) 30129–30134.
[13] K.H. Yip, H. Feng, N.J. Pavlos, M.H. Zheng, J. Xu, P62 ubiquitin binding-associated domain mediated the receptor activator of nuclear factor-kappaB ligand-induced osteoclast formation: a new insight into the pathogenesis of Paget's disease of bone, Am. J. Pathol. 169 (2006) 503–514.
[14] H. Feng, T. Cheng, J.H. Steer, D.A. Joyce, N.J. Pavlos, C. Leong, J. Kular, J. Liu, X. Feng, M.H. Zheng, J. Xu, Myocyte enhancer factor 2 and microphthalmia-associated transcription factor cooperate with NFATc1 to transactivate the V-ATPase d2 promoter during RANKL-induced osteoclastogenesis, J. Biol. Chem. 284 (2009) 14667–14676.
[15] A. Qin, T.S. Cheng, Z. Lin, L. Cao, S.M. Chim, N.J. Pavlos, J. Xu, M.H. Zheng, K.R. Dai, Prevention of wear particle-induced osteolysis by a novel V-ATPase inhibitor saliphenylhalamide through inhibition of osteoclast bone resorption, PLoS One 7 (2012) e34132.
[16] A. Qin, T.S. Cheng, Z. Lin, N.J. Pavlos, Q. Jiang, J. Xu, K.R. Dai, M.H. Zheng, Versatile roles of V-ATPases accessory subunit Ac45 in osteoclast formation and function, PLoS One 6 (2011) e27155.
[17] A. Qin, T.S. Cheng, N.J. Pavlos, Z. Lin, K.R. Dai, M.H. Zheng, V-ATPases in osteoclasts: structure, function and potential inhibitors of bone resorption, Int. J. Biochem. Cell. Biol. 44 (2012) 1422–1435.
[18] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method, Methods 25 (2001) 402–408.
[19] C. Wang, J.H. Steer, D.A. Joyce, K.H. Yip, M.H. Zheng, J. Xu, 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibits osteoclastogenesis by suppressing RANKL-induced NF-kappaB activation, J. Bone Miner. Res. 18 (2003) 2159–2168.
[20] A.C. Santos, P. Adkilen, The alkaloids of argemone mexicana, J. Am. Chem. Soc. 54 (1932) 2923–2924.
[21] B.J.R. Pitts, L.R. Meyerson, Inhibition of Na, K-ATPase activity and ouabain binding by sanguinarine, Drug Dev. Res. 1 (1981) 43–49.
[22] N. Aburai, M. Yoshida, M. Ohnishi, K. Kimura, Sanguinarine as a potent and specific inhibitor of protein phosphatase 2C in vitro and induces apoptosis via phosphorylation of p38 in HL60 cells, Biosci. Biotechnol. Biochem. 74 (2010) 548–552.
[23] M. Sun, W. Lou, J.Y. Chun, D.S. Cho, N. Nadiminty, C.P. Evans, J. Chen, J. Yue, Q. Zhou, A.C. Gao, Sanguinarine suppresses prostate tumor growth and inhibits survivin expression, Genes Cancer 1 (2010) 283–292.
[24] J. Holy, G. Lamont, E. Perkins, Disruption of nucleocytoplasmic trafficking of cyclin D1 and topoisomerase II by sanguinarine, BMC Cell Biol. 7 (2006) 13.
[25] N. Ahmad, S. Gupta, M.M. Husain, K.M. Heiskanen, H. Mukhtar, Differential antiproliferative and apoptotic response of sanguinarine for cancer cells versus normal cells, Clin. Cancer Res. 6 (2000) 1524–1528.
[26] L. Xing, T.P. Bushnell, L. Carlson, Z. Tai, M. Tondravi, U. Siebenlist, F. Young, B.F. Boyce, 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 (2002) 1200–1210.
[27] J. Xu, H.F. Wu, E.S. Ang, K. Yip, M. Woloszyn, M.H. Zheng, R.X. Tan, NF-kappaB modulators in osteolytic bone diseases, Cytokine Growth Factor Rev. 20 (2009) 7–17.
[28] S. Ghosh, M. Karin, Missing pieces in the NF-kappaB puzzle, Cell 109 (Suppl) (2002) S81–S96.
[29] B.F. Boyce, L. Xing, G. Franzoso, U. Siebenlist, Required and nonessential functions of nuclear factor-kappaB in bone cells, Bone 25 (1999) 137–139.
[30] G. Franzoso, L. Carlson, L. Xing, L. Poljak, E.W. Shores, K.D. Brown, A. Leonardi, T. Tran, B.F. Boyce, U. Siebenlist, Requirement for NF-kappaB in osteoclast and B-cell development, Genes Dev. 11 (1997) 3482–3496.
[31] V. Iotsova, J. Caamano, J. Loy, Y. Yang, A. Lewin, R. Bravo, Osteopetrosis in mice lacking NF-kappaB1 and NF-kappaB2, Nat. Med. 3 (1997) 1285–1289.