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Myristoleic acid inhibits osteoclast formation and bone resorption by suppressing the RANKL activation of Src and Pyk2

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ABSTRACT

Cytoskeletal changes in osteoclasts such as formation of actin ring is required for bone-resorbing activity. The tyrosine kinase Src is a key player in massive cytoskeletal change of osteoclasts, thereby in bone destruction. In order for Src to be activated, trafficking to the inner plasma membrane via myristoylation is of importance. A previous study reported that myristoleic acid derived from myristic acid, inhibited N-myristoyl-transferase, an essential enzyme for myristoylation process. This prompted us to investigate whether myristoleic acid could affect osteoclastogenesis. Indeed, we observed that myristoleic acid inhibited RANKL-induced osteoclast formation *in vitro*, especially, at later stages of differentiation. Myristoleic acid attenuated the tyrosine phosphorylation of c-Src and Pyk2, which associates with Src, by RANKL. When myristoleic acid was co-administered with soluble RANKL into mice, RANKL-induced bone loss was substantially prevented. Bone dissection clearly revealed that the number of multinucleated osteoclasts was significantly diminished by myristoleic acid. On the other hand, myristoleic acid treatment had little or no influence on early osteoclast differentiation markers, such as c-Fos and NFATc1, and proteins related to cytoskeletal rearrangement, including DC-STAMP, integrin αv and integrin β3 *in vitro*. Taken together, our data suggest that myristoleic acid is capable of blocking the formation of large multinucleated osteoclasts and bone resorption likely through suppressing activation of Src and Pyk2.

1. Introduction

Bone is a dynamic organ that is continuously remodeled by osteoclasts and osteoblasts (Karsenty et al., 2009). In bone remodeling, osteoclasts resorb bone matrix to remove bone areas which have structural defects or to maintain mineral homeostasis of the body. Osteoclasts are multinucleated cells generated from monocytes and macrophages in response to the differentiation factor RANKL (receptor activator of nuclear factor-κB ligand) in presence of macrophage colony-stimulating factor (M-CSF) that supports osteoclastogenesis by stimulating cell proliferation and survival during the differentiation (Boyle et al., 2003).

Uncontrolled expansion of osteoclasts, due to physiological changes such as menopause or inflammation leads to osteoporosis. In addition, osteoclasts seem to be deeply engaged in bone tumor metastasis (Rodan and Martin, 2000; Tanaka et al., 2005). Therefore, suppression of osteoclastogenesis is a key principle for therapeutics. Currently, bisphosphonate-derivatives and calcitonin are major therapeutics for osteoporosis, but they have apparent disadvantages such as being non-metabolized (Chesnut et al., 2008; Kyle et al., 2007). More recently, RANKL antibody hindering RANKL/RANK binding, the key signal of osteoclastogenesis, has begun being applied to osteoporosis. However, treatment costs and their long-term safety remain to be explored because RANKL is involved in dendritic cell function (Body et al., 2006). Hence, the necessity of novel therapeutic interventions is evident for these unmet medical needs.

In organs, some proteins need to be transferred from the cytosol to the plasma membrane for signal transduction or activation. Myristoylation of proteins approves membrane binding of the protein (Martin et al., 2011). Myristoylation is one of post-translational protein modifications, which refers to the covalent attachment of myristic acid (n-tetradecanoic acid) (Fig. 1A) to internal or N-terminal glycine residues of target proteins (Wright et al., 2010). Myristoylation of target proteins is catalyzed by N-myristoyl-transferase (NMT), a member of the GCN5-related *N*-acetyltransferase superfamily of proteins, which is conserved in eukaryotic organisms (Selvakumar et al., 2007). In cells, there are many proteins targeted by NMT including Src, calcineurin B and G-protein (Feng and Stemmer, 1999; Selvakumar et al., 2007). In case of Src, non-myristoylated Src remains in the cytoplasm in an inactive state, being unable to promote cytoskeleton rearrangements. Therefore, myristoylation is an important process for Src to

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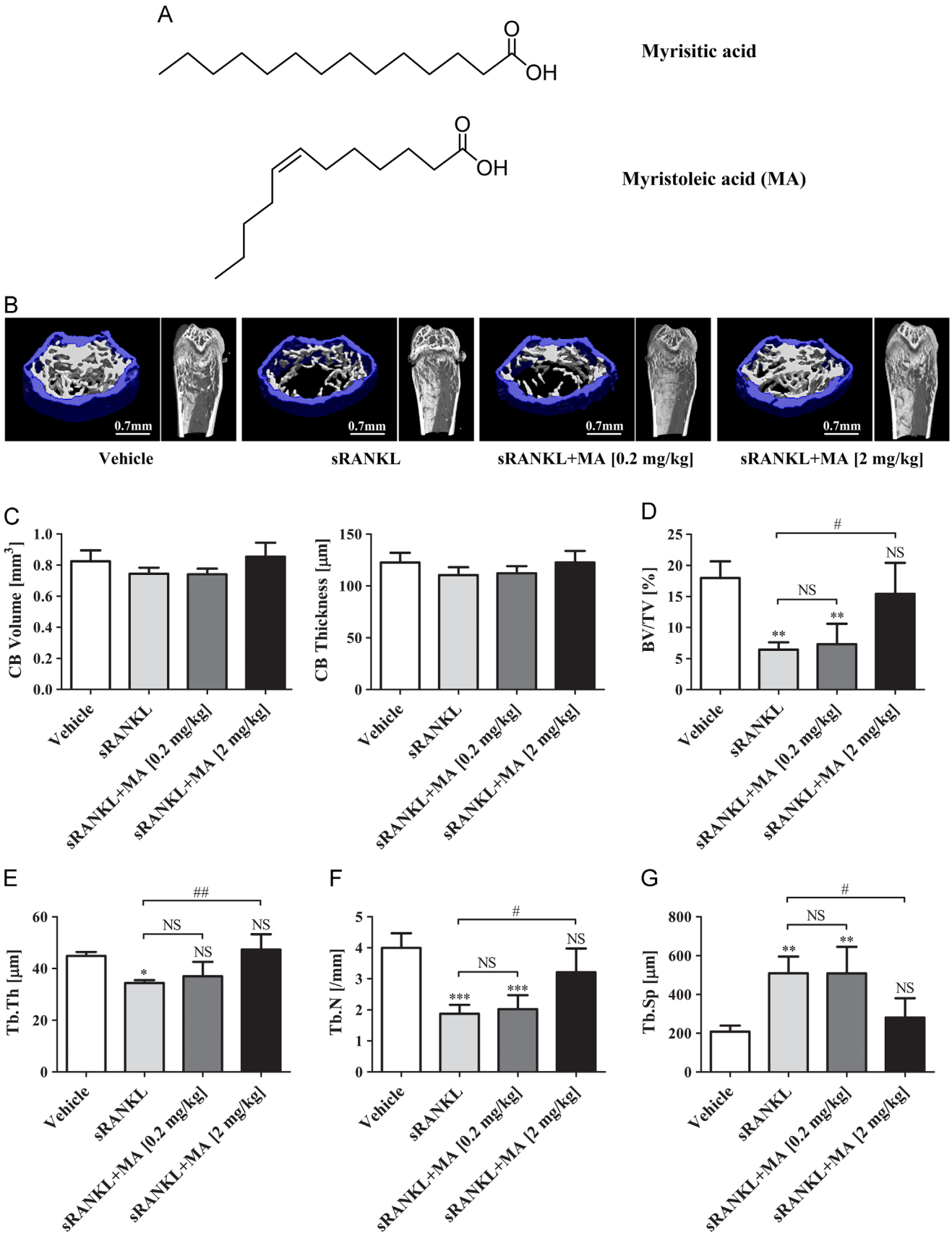

Fig. 1. Restoration of bone loss induced by sRANKL via co-administration of myristoleic acid. (A) Chemical structures of myristic acid and myristoleic acid (MA). (B–G) sRANKL-treated mice were administered with vehicle, 0.2 mg/kg and 2 mg/kg myristoleic acid. (B) Mice femurs were subjected to μCT analysis. Bone parameters, such as (C) cortical bone volume (CB volume), cortical bone thickness (CB thickness), (D) bone volume per tissue volume (BV/TV), (E) trabecular thickness (Tb.Th), (F) trabecular number (Tb.N) and (G) trabecular separation (Tb.Sp), were analyzed. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *versus* mice administrated with vehicle. $\#P < 0.05$ and $\#\#P < 0.01$ *versus* mice administrated with sRANKL only. NS, not significant.