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Inhibitory effects of mevastatin and a geranylgeranyl transferase I inhibitor (GGTI-2166) on mononuclear osteoclast formation induced by receptor activator of NFκB ligand (RANKL) or tumor necrosis factor-α (TNF-α)

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Abstract

We have previously reported that the statin mevastatin (compactin) reversibly inhibits the fusion of TRAP-positive mononuclear preosteoclasts (pOCs) into multinucleated osteoclasts and disrupts the actin ring in mature osteoclasts through the inhibition of protein prenylation. Protein geranylgeranylation, specifically, is known to be required for pOC fusion and for the function and survival of mature osteoclasts. However, it has not been determined whether protein geranylgeranylation is involved in early differentiation of osteoclasts (pOC formation). The current study shows that statins and the geranylgeranyl transferase I inhibitor GGTI-2166 inhibit the pOC formation induced by RANKL or TNF-α in cultures of both mouse marrow-derived macrophage-colony-stimulating factor (M-CSF) dependent monocytes (MD cells) and the mouse monocyte cell line RAW 264.7 (RAW cells). Mevastatin, 0.1–0.6 μM, inhibited the formation of pOCs induced by receptor activator of nuclear factor-κB ligand (RANKL) or tumor necrosis factor (TNF-α) in both cell cultures. The inhibitory effects of mevastatin were overcome by the addition of mevalonate, farnesyl pyrophosphate or geranylgeranyl pyrophosphate. GGTI-2166 inhibited TRAP activity induced by RANKL or TNF-α in both cell cultures and prevented the incorporation of [^{3}H]all-*trans* geranylgeraniol into prenylated proteins in RAW cells. However, the farnesyl transferase inhibitor FTI-2153 did not inhibit TRAP activity although FTI prevented the incorporation of [^{14}C]mevalonate into farnesylated proteins in RAW cells. *Clostridium difficile* cytotoxin B (toxin B) inhibited pOC formation induced by RANKL or TNF-α in both cell cultures. The inhibitory effects of statins and GGTI-2166 on pOC formation may result from the inhibition of the geranylgeranylation of G-proteins, such as Rho or Rac, suggesting that the geranylgeranylation of these proteins is involved in the early differentiation of progenitor cells into pOCs.

Keywords: Osteoclast differentiation; Statin; GGTI; FTI; Geranylgeranylation; Small G-protein

1. Introduction

Osteoclasts (OCL) are multinucleated cells (3–100 nuclei per cell) that play a critical role in bone remodeling. Osteoclasts are formed by the fusion of TRAP-positive mononuclear preosteoclasts (pOCs), which differentiate from hematopoietic precursors [1]. Osteoclasts, when activated, exhibit bone-resorbing activity [2–4].

Abbreviations: pOC, preosteoclast; RANKL, receptor activator of NFκB ligand; M-CSF, macrophage colony-stimulating factor; GGTI, geranylgeranyl transferase inhibitor; GGOH, geranylgeraniol; FTI, farnesyl transferase inhibitor; TRAP, tartrate-resistant acid phosphatase; TNF, tumor necrosis factor; GTP, guanosine triphosphate; HMG-CoA, hydroxymethylglutaryl coenzyme A; MEM, minimal essential medium; MD, marrow derived M-CSF dependent monocytes; MTT, methyl thiazole tetrazolium

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Small GTP-binding proteins (G-proteins) of the Ras superfamily (e.g., Ras, Rho, Rab, and cdc-42) play important roles in cell proliferation, differentiation, structural organization, and apoptosis [4–8]. The G-proteins Rab, Rho, and Rac are known to regulate the function of mature osteoclasts [9–12]. The activity of these proteins is regulated by a critical step in post-translational processing, the addition of isoprenoid farnesyl and geranylgeranyl groups [13–15].

Nitrogen-containing bisphosphonates inhibit multinucleated osteoclast formation, suppress osteoclastic bone resorption and induce apoptosis in mature osteoclasts [16–19]. Recently, nitrogen-containing bisphosphonates have been shown to prevent the production of isoprenyl diphosphates, such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), through inhibition of farnesyl diphosphate synthase, an enzyme in the cholesterol synthesis pathway [20]. FPP and GGPP are substrates for prenyl protein transferases that prenylate small GTP-binding proteins such as Rab, Rho, Rac, and cdc-42. Therefore, it has been suggested that the inhibitory effects of nitrogen-containing bisphosphonates on bone resorption result from the decrease in the prenylation of these proteins in mature osteoclasts. In other studies, the post-translational prenylation of small GTP-binding proteins was shown to be involved in the effects of nitrogen-containing bisphosphonates [16,21,22].

Statins, specific HMG-CoA reductase inhibitors that inhibit the synthesis of mevalonate, have similar effects on osteoclasts to those of nitrogen-containing bisphosphonates. We have shown previously that the statin mevastatin (compactin) reversibly inhibits the fusion of pOCs into multinucleated osteoclasts, disrupts the actin ring in mature osteoclasts, and suppresses bone-resorption activity without promoting apoptosis in the osteoclasts [23]. The inhibition of mevalonate synthesis also prevents the synthesis of its downstream intermediates, FPP and GGPP. Like the nitrogen-containing bisphosphonates, statins also exert these effects on osteoclasts by preventing the prenylation of small GTP-binding proteins. Recently, it has been shown that a geranylgeranyl transferase I inhibitor (GGTI), but not a farnesyl transferase inhibitor (FTI), inhibits the formation, function and survival of multinucleated osteoclasts in bone marrow cultures [16]. These findings suggest that the inhibitory effect of nitrogen-containing bisphosphonates and statins on osteoclasts result from the decrease of geranylgeranylation of prenylated G-proteins.

Although statins and GGTI inhibit OCL formation in bone marrow cell cultures or cocultures of bone marrow cells and osteoclasts/stromal cells, it has not been determined whether statins and GGTI affect the differentiation of osteoclast precursor cells into pOCs. In this study, we determined the effects of mevastatin, GGTI-2166 (GGTase I specific inhibitor), and FTI-2153 (FTase specific inhibitor) on pOC formation induced by receptor activator of NF-κB ligand (RANKL) or tumor necrosis factor-α (TNF-α) in cultures of mouse marrow-derived M-CSF dependent monocytes (MD cells) or the mouse monocyte cell line RAW 264.7 (RAW cells). We show that statins and GGTI inhibit pOC formation induced by RANKL or TNF-α in both cell culture models. Furthermore, we show that *Clostridium difficile* cytotoxin B (toxin B), which inactivates small Rho family proteins such as Rac, Rho and cdc-42, inhibits the effect of RANKL or TNF-α to induce early differentiation of these two types of cells into pOCs. The results suggest that the inhibitory effects of statins and GGTI on bone resorption are partially due to the inhibition of pOC formation, and that geranylgeranylation of G-proteins such as Rho or Rac may be required for pOC formation.

2. Materials and methods

2.1. Materials

Farnesyl pyrophosphate, geranylgeranyl pyrophosphate, squalene, fast violet LB salt and naphthol AS-MX phosphate were purchased from Sigma Chemical Co. Mevalonic acid lactone was purchased from Wako Pure Chemicals Co. Mevastatin (compactin) was a generous gift from Dr. A. Endo (Biopharm Research Laboratories). Lovastatin and simvastatin were generous gifts from Sankyo Co. Recombinant murine M-CSF and recombinant human RANKL (sRANKL) were from R&D Systems and Pepro Tech EC Ltd., respectively. Recombinant murine TNF-α was purchased from R&D Systems. [^{3}H]all-*trans* geranylgeraniol (GGOH) and [^{14}C]mevalonolactone were from Amersham Radiochemicals Ltd. All other reagents were obtained from Sigma unless otherwise stated.

2.2. Cell culture

The mouse monocyte cell line RAW 264.7 (RAW cells) was obtained from American Type Culture Collection. The cells were cultured in Dulbecco's modified Eagle's medium (GIBCO) containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 mM L-glutamine (α-MEM + 10% FBS) at 37 °C in a humidified atmosphere of 5% CO_2 in air.

2.3. Preparation of MD cells from bone marrow cells

Bone marrow cells were isolated from 5 to 7-day-old CD-1 mice as described previously [24]. Tibiae and femora were aseptically removed and dissected free of adherent soft tissue. The bone ends were cut off and the marrow was forced out into a petri dish containing α-MEM + 10% FBS. The marrow suspension was filtered with a fine mesh sieve to remove bone particles and carefully agitated with a plastic Pasteur pipette to obtain a single-cell suspension. The bone marrow cells were washed twice, resuspended in α-MEM + 10% FBS, and incubated for 24 h in M-CSF