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Review

Lysophosphatidic acid: A potential mediator of osteoblast–osteoclast signaling in bone ☆

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ABSTRACT

Osteoclasts (bone resorbing cells) and osteoblasts (bone forming cells) play essential roles in skeletal development, mineral homeostasis and bone remodeling. The actions of these two cell types are tightly coordinated, and imbalances in bone formation and resorption can result in disease states, such as osteoporosis. Lysophosphatidic acid (LPA) is a potent bioactive phospholipid that influences a number of cellular processes, including proliferation, survival and migration. LPA is also involved in wound healing and pathological conditions, such as tumor metastasis and autoimmune disorders. During trauma, activated platelets are likely a source of LPA in bone. Physiologically, osteoblasts themselves can also produce LPA, which in turn promotes osteogenesis. The capacity for local production of LPA, coupled with the proximity of osteoblasts and osteoclasts, leads to the intriguing possibility that LPA acts as a paracrine mediator of osteoblast–osteoclast signaling. Here we summarize emerging evidence that LPA enhances the differentiation of osteoclast precursors, and regulates the morphology, resorptive activity and survival of mature osteoclasts. These actions arise through stimulation of multiple LPA receptors and intracellular signaling pathways. Moreover, LPA is a potent mitogen implicated in promoting the metastasis of breast and ovarian tumors to bone. Thus, LPA released from osteoblasts is potentially an important autocrine and paracrine mediator — physiologically regulating skeletal development and remodeling, while contributing pathologically to metastatic bone disease. This article is part of a Special Issue entitled Advances in Lysophospholipid Research.

1. Introduction

The actions of osteoclasts (bone-resorbing cells) and osteoblasts (bone-forming cells) are vital for skeletal development and remodeling [1,2]. Bone cells continuously receive signals from adjacent cells, soluble mediators and the extracellular matrix to regulate their proliferation, activity and survival. Balance between resorption and formation is critical for skeletal homeostasis, and imbalance can lead to diseases such as osteoporosis [3,4].

Lysophosphatidic acid (LPA) is a potent bioactive phospholipid, present at low levels in plasma (~100 nM) [5] and elevated levels at sites of tissue injury and inflammation [5,6]. Produced by several cell types including activated platelets, LPA signals through at least five well-described G protein-coupled receptors, LPA1–LPA5, each of which can couple to multiple heterotrimeric G proteins [7,8]. Downstream responses can include elevation of cytosolic free calcium concentration ($[Ca^{2+}]_i$), activation of Ras and extracellular signal-regulated kinases (ERK), and stimulation of phosphatidylinositol 3-kinase (PI3K)/Akt signaling [9]. Moreover, LPA receptors couple to Rho and Rac to elicit changes in cytoskeletal organization, thereby regulating cell migration and chemotaxis [10].

The actions of LPA on osteoblasts are described in detail in another article in this Special Issue. In brief, LPA acts to increase DNA synthesis and induce chemotaxis through interactions with the LPA1 receptor [11]. In addition, LPA acts on osteoblastic cells to induce the synthesis of cytokines [12], which are known to regulate osteoclast behavior. Recently, our work has revealed that osteoblasts are a source of LPA production in bone, which may regulate osteoclast functions and contribute to the pathogenesis of bone diseases. For example, LPA, which is elevated in the synovial fluid of patients with rheumatoid

Abbreviations: BEL, bromoenol lactone; BzATP, 2′,3′-*O*-(4-benzoylbenzoyl)-ATP; $[Ca^{2+}]_i$, cytosolic free calcium concentration; ERK, extracellular signal-regulated kinase; F-actin, filamentous actin; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPG, lysophosphatidylglycerol; M-CSF, macrophage colony stimulating factor; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor κB; PA, phosphatidic acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI3K, phosphatidylinositol 3-kinase; PLA_2, phospholipase A_2; PLD, phospholipase D; PAF, platelet-activating factor; RANKL, receptor activator of nuclear factor κB ligand; TRIP6, thyroid receptor-interacting protein 6

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arthritis, stimulates the migration of synoviocytes and the production of inflammatory cytokines, implicating this lysophospholipid in the progression of rheumatoid arthritis [13,14]. In this review, we will describe a conceptual framework in which LPA acts as a mediator linking osteoblasts to osteoclasts in healthy and disease states.

2. P2X7 receptors initiate production of LPA in osteoblasts through activation of phospholipases

To explain the evidence that osteoblasts can produce LPA, we will first provide some background on nucleotide receptors and nucleotide signaling in bone. Nucleotides such as ATP are thought to be key mediators in the response of bone cells to mechanical stimuli [15–18]. Many cells release ATP into the extracellular fluid when subjected to stretch or fluid shear [19–21]. Extracellular ATP can then signal through P2 nucleotide receptors present on various cell types including osteoblasts. There are two subtypes of P2 receptors, P2X (ligand-gated cation channels) and P2Y (G protein-coupled receptors) [22]. Osteoblasts express multiple subtypes of P2X and P2Y receptors [16,23,24], and the mechanistic bases of nucleotide effects on bone are now emerging.

P2X7 receptors function not only as non-selective cation channels, but can also induce the formation of large membrane pores, which allow passage of molecules with size up to 900 Da [25,26]. Pore formation may arise from dilation of the channel due to recruitment of additional subunits, while an alternative proposal is that P2X7 receptor activation leads to opening of a distinct pore protein in the plasma membrane [27].

P2X7 receptors have been implicated in skeletal remodeling and mechanotransduction. Mice lacking functional P2X7 receptors (*P2rx7*$^{-/-}$ mice) exhibit decreased periosteal bone formation in long bones without a difference in length [28]. Moreover, *P2rx7*$^{-/-}$ mice show reduced osteogenesis in response to mechanical loading [29]. Activation of P2X7 receptors induces dynamic membrane blebbing in a subpopulation of calvarial cells and LPA has been identified as a key element in this signaling pathway [30].

Relevant to the topic of this review, Panupinthu et al. went on to use a biochemical approach to directly investigate whether P2X7 receptor activation caused production of LPA [31]. Rat calvarial cells were labeled with [^{14}C]glycerol overnight and then incubated with of 2′,3′-*O*-(4-benzoylbenzoyl)-ATP (BzATP, a relatively potent P2X7 agonist, 300 μM) or vehicle (Control) for 20 min. Cells were collected and total lipids were separated using thin layer chromatography (Fig. 1A). BzATP induced a significant increase in levels of phosphatidic acid (PA, Fig. 1*Bi*). The phospholipase D (PLD) inhibitor 1-butanol (1% v/v) completely inhibited the production of phosphatidic acid induced by BzATP. In contrast, its inactive analog *tert*-butanol had little effect. Moreover, the PLA_2 inhibitor bromoenol lactone (BEL, 10 μM) had no significant effect on phosphatidic acid synthesis. BzATP also stimulated production of LPA itself (Fig. 1*Bii*). This effect was abolished by 1-butanol as well as by BEL, whereas *tert*-butanol had no significant effect. In the absence of phospholipase inhibitors, BzATP caused a decrease in levels of phosphatidylcholine (PC, Fig. 1*Biii*), consistent with phosphatidylcholine being a preferred substrate of PLD [32]. Similar results were found in studies of murine osteoblasts, and importantly, BzATP failed to induce LPA production in cells from *P2rx7*$^{-/-}$ mice [31]. These findings established directly that activation of P2X7 receptors in osteoblasts leads to production of the LPA, through the sequential activation of PLD and PLA_2.

This observation suggests the possibility that LPA, produced in osteoblasts by stimulation of P2X7 receptors, acts in an autocrine manner to regulate osteoblast activity [30,31]. Moreover, osteoblast-derived LPA may act as a paracrine factor to regulate the activity of tumor cells in the skeletal microenvironment as well as osteoclasts (Fig. 2). Other articles in this Special Issue review the effects of LPA on

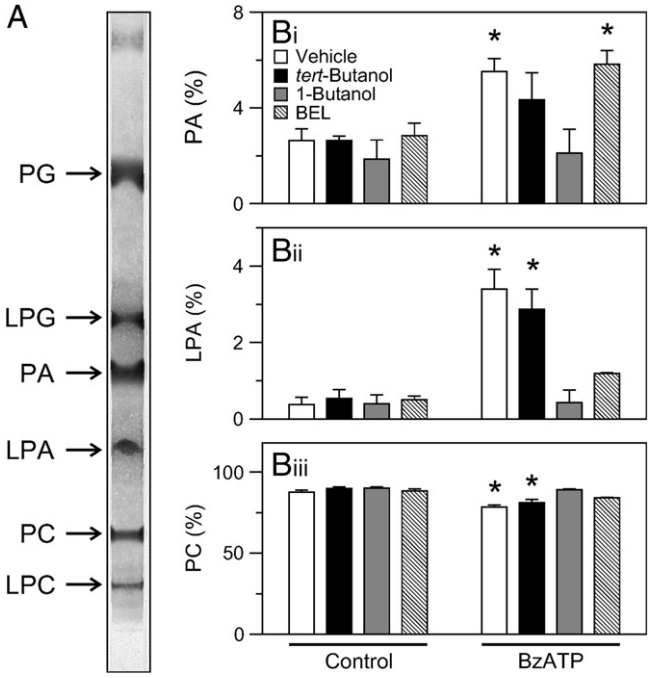

Fig. 1. Osteoblasts produce LPA in response to stimulation of P2X7 receptors. A, Representative thin layer chromatograph illustrating reference lipids: PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PA, phosphatidic acid; LPA, lysophosphatidic acid; PC, phosphatidylcholine; and LPC, lysophosphatidylcholine (phosphatidylethanolamine was omitted for clarity). B, Rat calvarial cells were labeled with 1 μCi/ml [^{14}C]glycerol at 37 °C for 18 h. Cells were then incubated for 10 min at 37 °C with 1-butanol (PLD inhibitor, 1% v/v), *tert*-butanol (inactive analog of 1-butanol, 1% v/v), BEL (PLA_2 inhibitor, 10 μM), or vehicle. BzATP (300 μM) or its vehicle (Control) was then added and cultures were incubated for an additional 20 min in the continued presence of inhibitors. Lipids were extracted from the cell layer and separated by thin layer chromatography. The percentages of radioactivity in [^{14}C]PA, [^{14}C]LPA and [^{14}C]PC were determined. *Indicates significant difference compared with the corresponding Control ($p<0.05$, $n=3$ independent preparations). These data reveal that P2X7 receptors can activate PLD and PLA_2 leading to production of LPA by osteoblasts.
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osteoblasts and tumor cells in bone. Below, we review our emerging understanding of the effects of LPA on osteoclasts.

3. LPA receptor types in osteoclasts

Isolation of authentic osteoclasts from long bones yields a mixed cell preparation that includes mature osteoclasts and their precursors, as well as bone marrow stromal cells. Accordingly, to characterize LPA receptor expression in a purified preparation, Lapierre et al. differentiated murine bone marrow precursors in vitro and purified osteoclasts from these preparations [33]. Real-time RT-PCR revealed that LPA1 was the predominant LPA receptor, with transcript levels ~20% of those of the calcitonin receptor, a G protein-coupled receptor that is expressed selectively and at high levels in osteoclasts [34]. Lower levels of transcripts encoding LPA2, LPA4 and LPA5 were present, whereas LPA3 message was not detectable [33]. These findings are in keeping with the expression pattern of LPA receptors in human monocytes – precursors of osteoclasts – in which LPA1 and LPA2, but not LPA3 were detected by immunoblot [35].

4. LPA elicits elevations of cytosolic free calcium concentration in osteoclasts

To investigate whether LPA stimulates elevation of $[Ca^{2+}]_i$, Lapierre et al. loaded freshly isolated rat and rabbit osteoclasts with the Ca^{2+}-sensitive fluorescent probe fura-2 and monitored single