Biochemical and Biophysical Research Communications 443 (2014) 694–699

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

A supra-cellular model for coupling of bone resorption to formation during remodeling: lessons from two bone resorption inhibitors affecting bone formation differently ☆

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ARTICLE INFO

Article history:
Received 3 December 2013
Available online 12 December 2013

Keywords:
Coupling
Osteoclast anabolics
Osteoprogenitors
Bone remodeling compartment canopy
Cathepsin K inhibitor
Bisphosphonates

ABSTRACT

The bone matrix is maintained functional through the combined action of bone resorbing osteoclasts and bone forming osteoblasts, in so-called bone remodeling units. The coupling of these two activities is critical for securing bone replenishment and involves osteogenic factors released by the osteoclasts. However, the osteoclasts are separated from the mature bone forming osteoblasts in time and space. Therefore the target cell of these osteoclastic factors has remained unknown. Recent explorations of the physical microenvironment of osteoclasts revealed a cell layer lining the bone marrow and forming a canopy over the whole remodeling surface, spanning from the osteoclasts to the bone forming osteoblasts. Several observations show that these canopy cells are a source of osteoblast progenitors, and we hypothesized therefore that they are the likely cells targeted by the osteogenic factors of the osteoclasts. Here we provide evidence supporting this hypothesis, by comparing the osteoclast-canopy interface in response to two types of bone resorption inhibitors in rabbit lumbar vertebrae. The bisphosphonate alendronate, an inhibitor leading to low bone formation levels, reduces the extent of canopy coverage above osteoclasts. This effect is in accordance with its toxic action on periosteoclastic cells. In contrast, odanacatib, an inhibitor preserving bone formation, increases the extent of the osteoclast-canopy interface. Interestingly, these distinct effects correlate with how fast bone formation follows resorption during these respective treatments. Furthermore, canopy cells exhibit uPARAP/Endo180, a receptor able to bind the collagen made available by osteoclasts, and reported to mediate osteoblast recruitment. Overall these observations support a mechanism where the recruitment of bone forming osteoblasts from the canopy is induced by osteoclastic factors, thereby favoring initiation of bone formation. They lead to a model where the osteoclast-canopy interface is the physical site where coupling of bone resorption to bone formation occurs.

1. Introduction

Bone remodeling consists of bone resorption by osteoclasts followed by bone formation by osteoblasts. The mechanism ensuring the restoration of resorbed bone is gaining increasing attention because malfunction of this mechanism contributes to bone loss and fractures. An important concept is that osteoclasts are part of this mechanism, probably through the release of pro-osteoblastic factors [1–3]. The basis of this concept is that general inhibitors of osteoclasts, such as alendronate (ALN), a bisphosphonate, lead to decreased bone formation, whereas inhibition restricted to their resorptive activity sustains or even increases bone formation [2,4,5] while increasing the number of non-resorbing osteoclasts [6–9]. An example of the latter inhibitors is odanacatib (ODN), a selective inhibitor of cathepsin K, the main proteinase degrading collagen during bone resorption. This compound is presently in a phase III clinical trial for the treatment of osteoporosis.

Importantly, osteoclasts and bone forming osteoblasts are separated in time and space during the remodeling cycle, and it is still not understood how the osteoclast-derived factors make osteoblasts restitute locally the bone matrix [1]. We previously

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http://dx.doi.org/10.1016/j.bbrc.2013.12.036

proposed that osteoclast osteogenic products should target cells in the immediate osteoclast surroundings, thereby promoting the reversal phase of the remodeling cycle, which is critical for recruitment of osteoprogenitors and initiation of bone formation [10,11]. In line with this hypothesis, we found that ODN induces a shorter reversal phase, higher osteoblast recruitment, and an increase in osteoclast surface in ovariectomized rabbits, whereas ALN did not show these responses [7]. Accordingly, a previous analysis of the same rabbits, showed that ODN had a positive effect on bone formations rate, whereas ALN decreased bone formation rates [5]. Amongst the cells which may be targeted by osteoclasts are reversal cells on the bone surface [7,10,11], canopy cells at the interface of the bone marrow and the bone remodeling site [12–14], and vasculature-associated cells at the canopy-marrow interface [12,15]. All belong to the osteoblast lineage and may serve as progenitors of bone forming osteoblasts [11]. Particular attention on the likely participation of canopy cells in this process is drawn by decreased bone formation in disease situations of canopy deficiency [12,14,16]. Here we hypothesized that canopy cells might be the unidentified partners of the osteoclasts, allowing them to exert their anabolic role. We tested this hypothesis by extending the analyses of our earlier study performed in ovariectomized rabbits, where the extent of osteoclasts surfaces, the reversal phase, and bone formation were promoted by ODN, but not by ALN [5,7]. The question we asked was whether the distinct effects of ODN and ALN we reported on these parameters would coincide with distinct effects of ODN and ALN on the osteoclast-canopy interface, thereby suggesting a causal relationship.

2. Materials and methods

2.1. Immunohistochemistry, histomorphometry and electron microscopy

The present study is a follow-up of our recent study reporting the effects of ALN and ODN on post-osteoclastic events in ovariectomized rabbits [7]. We used the same lumbar vertebrae from the four experimental groups: sham-operated, ovariectomized treated with vehicle, ALN, or ODN [7]. For immunohistochemical staining, paraffin sections (3.5 μm thick) from the second lumbar vertebrae were processed as described [7]. Immunostaining for the endocytic collagen receptor urokinase plasminogen activator receptor-associated protein (uPARAP/Endo 180), was performed using a monoclonal mouse antibody, 2h9F12, [17] which was detected with a polymeric alkaline phosphatase conjugated system (Bright Vision, Immunologic, Duiven, Holland) and visualized by liquid permanent red (DakoDenmark A/S, Glostrup, Denmark). Negative controls were performed by using an isotype-matched mouse control immunoglobulin (IgG1, MOPC-21, Ab18443, Abcam). Sections were counterstained with Mayer's haematoxylin and mounted.

Histomorphometric parameters were assessed in the trabecular bone of Masson-Goldner trichrome-stained sections (6 μm thick) prepared from the plastic-embedded fourth lumbar vertebrae as described by Jensen et al. [7]. The parameters included the proportion of bone surface covered by osteoclast (Oc.S), reversal (Rv.S), and osteoid surface (OS), where each parameter was determined in relation to the presence or absence of a canopy [12,14–16]. Reversal surfaces were defined as eroded surfaces without osteoclasts. Eroded surfaces were identified through visualization of broken lamellae in polarized light. Canopies were defined as a continuous layer of elongated cells lining the bone marrow and separated from the bone matrix by osteoclasts, reversal cells, or osteoblasts, and sometimes by a lumen [12,13] (Fig. 1A, yellow arrowheads). For every single hit on reversal perimeters, the presence of both an osteoclast and osteoid in the vicinity was recorded. Vicinity was defined as being within the same 2D remodeling unit as the reversal surface itself [7]. All measurements were done blinded with respect to sham, OVX, ALN, and ODN treatment.

Samples for electron microscopy were prepared and analyzed as previously described [15].

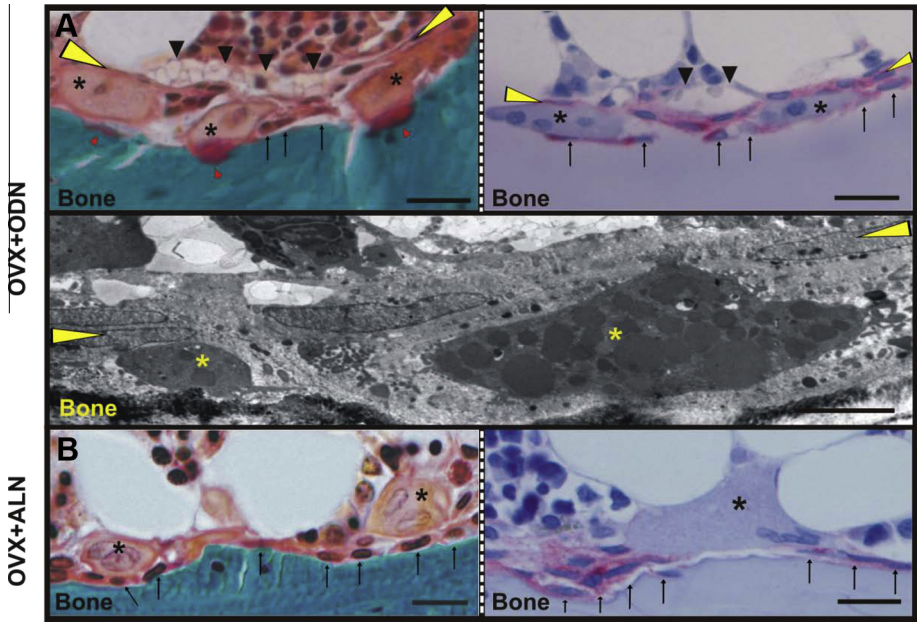

Fig. 1. Proximity of osteoclasts and canopy cells as it appears in histological sections. (A) Association between osteoclasts (*asterisk*) and canopy cells (*yellow arrowheads*) in the vertebral trabecular bone of ODN-treated OVX rabbits as it appears by using Masson-Goldner trichrome (*upper left*), uPARAP immunohistochemical staining (red) (*upper right*), and electron microscopy (*lower*). Note the close proximity of vascular structures (*black arrowheads*) and canopies (*upper panels*). (B) The association between osteoclasts (*asterisk*), and canopy cells is frequently lost in ALN-treated OVX rabbits as illustrated by Masson-Goldner trichrome (*left*) and uPARAP immunohistochemical staining (*right*). Arrows in (A) and (B) indicate reversal cells. Scale bar: 20 μm, except for *A, lower panel*: 2 μm.