Bone 73 (2015) 16–23

Contents lists available at ScienceDirect

Bone

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

Original Full Length Article

A joined role of canopy and reversal cells in bone remodeling — Lessons from glucocorticoid-induced osteoporosis

Pia Rosgaard Jensen [a,*], Thomas Levin Andersen [a], Ellen-Margrethe Hauge [b], Jens Bollerslev [c,d], Jean-Marie Delaissé [a]

[a] Department of Clinical Cell Biology (KCB), Vejle Hospital, IRS, University of Southern Denmark, Kabbeltoft 25, 7100 Vejle, Denmark
[b] Department of Rheumatology, Aarhus University Hospital, Building 3, Nørrebrogade 44, 8000 Aarhus C, Denmark
[c] Section of Specialized Endocrinology, Medical Clinic B, Oslo University Hospital, Oslo, Norway
[d] Faculty of Medicine, University of Oslo, Oslo, Norway

ARTICLE INFO

Article history:
Received 4 June 2014
Revised 19 September 2014
Accepted 4 December 2014
Available online 10 December 2014

Edited by: Moustapha Kassem

Keywords:
Bone remodeling
Glucocorticoid
Reversal phase
Bone remodeling compartment canopy
Coupling

ABSTRACT

Successful bone remodeling demands that osteoblasts restitute the bone removed by osteoclasts. In human cancellous bone, a pivotal role in this restitution is played by the canopies covering the bone remodeling surfaces, since disruption of canopies in multiple myeloma, postmenopausal- and glucocorticoid-induced osteoporosis is associated with the absence of progression of the remodeling cycle to bone formation, i.e. uncoupling. An emerging concept explaining this critical role of canopies is that they represent a reservoir of osteoprogenitors to be delivered to reversal surfaces. In postmenopausal osteoporosis, this concept is supported by the coincidence between the absence of canopies and scarcity of cells on reversal surfaces together with abortion of the remodeling cycle. Here we tested whether this concept holds true in glucocorticoid-induced osteoporosis. A histomorphometric analysis of iliac crest biopsies from patients exposed to long-term glucocorticoid treatment revealed a subpopulation of reversal surfaces corresponding to the characteristics of arrest found in postmenopausal osteoporosis. Importantly, these arrested reversal surfaces were devoid of canopy coverage in almost all biopsies, and their prevalence correlated with a deficiency in bone forming surfaces. Taken together with the other recent data, the functional link between canopies, reversal surface activity, and the extent of bone formation surface in postmenopausal- and glucocorticoid-induced osteoporosis, supports a model where bone restitution during remodeling demands recruitment of osteoprogenitors from the canopy onto reversal surfaces. These data suggest that securing the presence of functional local osteoprogenitors deserves attention in the search of strategies to prevent the bone loss that occurs during bone remodeling in pathological situations.

1. Introduction

Bone remodeling replaces existing bone by new bone through coordinated activities of bone resorbing osteoclasts and bone forming osteoblasts, which are organized in basic multicellular units (BMUs). Many bone diseases are due to disorders in bone remodeling. Typical examples are postmenopausal and glucocorticoid-induced osteoporosis. In both these diseases, osteoblast bone formation is insufficient compared to osteoclast resorption, thereby resulting in loss of bone mass and structure, and increased fracture risk [1]. The research focus for getting a better insight in this pathogenesis has mainly been the molecular regulation of osteoclast and osteoblast activities, and more recently, their coupling mechanism [2]. This has allowed the identification of a series of systemic and local factors involved in regulating the development and function of osteoclasts and osteoblasts, which include hormones, cytokines, growth and transcription factors [3]. However, the distance separating osteoclasts and bone forming osteoblasts stresses that understanding the coupling mechanism requires also to consider the cellular events which occur in this interval, and which are expected to support recruitment of osteoblasts. Histological observations of the BMU show that osteoclastic bone resorption is physically linked to bone formation through two bridging structures made of osteoblast-lineage cells [4]. The first one is known as the reversal surface. This surface is defined as an eroded bone surface vacated by osteoclasts and colonized by osteoblastic cells, which are called reversal cells [5–7]. The second one consists of a canopy of elongated osteoblast-lineage cells, covering the whole bone remodeling area, and separating it from the bone marrow [8,9].

Evidence for an effective involvement of these two structures in coupling osteoclastic bone resorption and osteoblastic bone formation originates from observations showing deficient bone formation when these structures appear to have a compromised bridging ability. Thus

* Corresponding author at: Department of Clinical Cell Biology (KCB), Vejle Hospital, Kabbeltoft 25, 7100 Vejle, Denmark. Fax: +45 79406864.
E-mail address: pia.rosgaard.jensen@rsyd.dk (P.R. Jensen).

http://dx.doi.org/10.1016/j.bone.2014.12.004
8756-3282/

canopy coverage is markedly lacking when bone formation is deficient in diseases such as multiple myeloma, endogenous Cushing's syndrome, postmenopausal osteoporosis, and during aging, compared to when resorption and formation are perfectly coupled, such as in primary hyperparathyroidism (PHPT) and healthy controls [8,10–12]. Similarly, the so-called "arrested" reversal surfaces were reported to be present in osteopenic situations [13]. These surfaces were characterized as sparsely populated by flat cells, resembling bone lining cells of quiescent surfaces [14]. Arrested reversal surfaces were further characterized in a recent study on postmenopausal osteoporosis, but could not be detected in PHPT [5].

An interesting hypothesis arising from the association of deficient bone formation with respectively lack of canopy coverage and reversal surface arrest is that there might also be an association between lack of canopy coverage and reversal surface arrest. In this respect, it is of note that these two bridging structures are made of osteoblast-lineage cells at different differentiation stages [12]. Canopy cells are at an earlier differentiation stage and three times more proliferative, compared to reversal cells [12]. Furthermore, early reversal cells occurring next to osteoclasts are at an earlier differentiation stage than the late cells detected next to osteoid, and bone formation is only initiated above a critical cell density [5,12]. These observations have lead to a model where canopies consist of a reservoir of osteoprogenitors that are recruited onto the reversal surfaces, where they progressively differentiate into mature bone forming osteoblasts [12]. Thus this model actually shows a functional link between lack of canopies, compromised reversal cell recruitment, scarcity of reversal cells, meaning arrested reversal phase, and the absence of initiation of bone formation. The existence of this functional link was supported in a recent study conducted on postmenopausal osteoporosis, where degree of canopy deficiency, extent of arrested reversal surface, and bone formation deficiency correlated with each other [10].

We reasoned that if this functional link represents a basic mechanism in human bone pathophysiology and not merely a peculiarity of postmenopausal osteoporosis, it should also operate in other situations. The present study addressed this issue in glucocorticoid (GC)-induced osteoporosis, a distinct pathophysiological situation, where insufficient bone formation may partially result from a lack of osteoprogenitors [15]. Therefore, we compared the putative osteoprogenitor recruitment sites in bone biopsies from a cohort of patients who were treated with high doses of GCs, with those from a cohort of matching controls. Correlations between canopy loss and deficient bone formation were already reported in endogenous Cushing's syndrome [11], a situation characterized by high endogenous levels of GCs. The present study investigated whether postmenopausal women on long-term GC therapy (exogenous Cushing's syndrome) show arrested reversal surfaces, what the prevalence of these surfaces is, and whether their presence was related with canopy coverage and bone formation.

2. Materials and methods

2.1. Patients and bone specimens

For histomorphometric analyses, 7-mm plastic-embedded transiliac crest bone biopsies were obtained from a total of 15 postmenopausal women (mean age of 69 ± 6 years) receiving GC therapy for between 1 and 17 years (mean duration of therapy: 6.7 years). The patients were prescribed 5 to 60 mg daily of prednisone (n = 14) or prednisolone (n = 1) (average prescribed daily dose: 14.7 mg) for treatment of the underlying diseases which included asthma (n = 6), temporal arthritis (n = 1), arthritis (n = 3), dermatitis (n = 1), polymyalgia (n = 3) and collagenosis (n = 1). All patients had experienced at least one low-energy vertebral fracture. Controls consisted of 7-mm plastic-embedded transiliac crest bone biopsies originating from 10 healthy postmenopausal volunteers with no history or physical signs of metabolic bone disease (mean age of 66 ± 9 years), and which were already used as controls in earlier studies [5,10,16]. Before biopsy removal, all patients and controls were submitted to tetracycline double labeling with a labeling interval of 10 days. The study was approved by the Danish National Committee on Biomedical Research Ethics (project S-20070121) and informed consent was obtained from all individuals included in the study.

2.2. Histomorphometry

Histomorphometric analyses were performed on Masson's trichrome-stained 7-μm thick sections, as described [11]. Standard histomorphometric parameters were estimated, including the proportion of cancellous bone surface (BS) covered by eroded (ES), osteoclast (Oc.S), reversal (Rv.S), osteoid (OS), and osteoblast surface (Ob.S). Based on cell morphology, osteoblast surfaces were divided into surfaces with cuboidal osteoblasts (C.Ob.S) and surfaces with flat osteoblasts (F.Ob.S) as described [17]. Reversal surfaces were defined as eroded surfaces vacated by osteoclasts, where eroded surfaces were identified through visualization of broken lamellae in polarized light. Reversal surfaces never remain cell-free and are colonized by elongated mononucleated cells with flattened nuclei, right after the departure of the osteoclast [4,18]. These cells are called reversal cells [5,6], and were recently demonstrated to be osteoblast-lineage cells preparing the bone surface for bone formation and differentiating into bone-forming osteoblasts [4,5,12,18,19]. A more detailed analysis was conducted on the reversal surfaces as previously described [5], subdividing them into active reversal surface (Ac.Rv.S) and arrested reversal surface (Ar.Rv.S). The former was defined as reversal surface flanked by an osteoclast and/or osteoid, whereas the latter was defined as reversal surface without neighboring osteoclast and osteoid. For each parameter mentioned above, the extent of canopy coverage was evaluated. Canopies were defined as a continuous layer of elongated cells lining

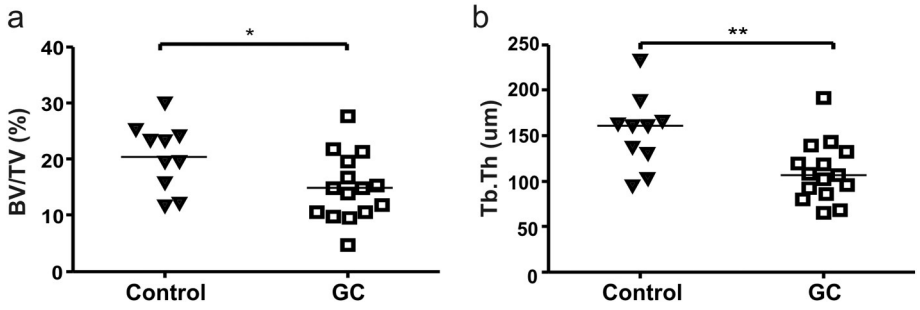

Fig. 1. Bone status in controls and GC-treated patients. The trabecular bone volume (BV/TV) (a) and the trabecular thickness (Tb.Th) (b) were estimated in controls (*triangles*) and GC-treated patients (*squares*). Horizontal bars indicate the mean values. The unpaired *t*-test was used to analyze whether these parameters were significantly different in the two populations. *$p < 0.05$ and **$p < 0.01$.