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Nitric oxide and cyclic GMP functions in bone

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ABSTRACT

Nitric oxide plays a central role in the regulation of skeletal homeostasis. In cells of the osteoblastic lineage, NO is generated in response to mechanical stimulation and estrogen exposure. Via activation of soluble guanylyl cyclase (sGC) and cGMP-dependent protein kinases (PKGs), NO enhances proliferation, differentiation, and survival of bone-forming cells in the osteoblastic lineage. NO also regulates the differentiation and activity of bone-resorbing osteoclasts; here the effects are largely inhibitory and partly cGMP-independent. We review the skeletal phenotypes of mice deficient in NO synthases and PKGs, and the effects of NO and cGMP on bone formation and resorption. We examine the roles of NO and cGMP in bone adaptation to mechanical stimulation. Finally, we discuss preclinical and clinical data showing that NO donors and NO-independent sGC activators may protect against estrogen deficiency-induced bone loss. sGC represents an attractive target for the treatment of osteoporosis.

1. Introduction

The appendicular skeleton and vertebrae develop via a process called endochondral ossification, where mesenchymal cells differentiate into chondroblasts to form a cartilaginous “template” that is replaced by osteoblasts producing mineralizing matrix [1]. After birth, endochondral ossification continues in the growth plates, where it governs longitudinal bone growth. Throughout the organismal lifespan, mineralized bone undergoes constant remodeling to maintain skeletal homeostasis and strength: osteoclasts initiate the remodeling cycle by resorbing mineralized matrix, while osteoblasts form new matrix (Fig. 1). An imbalance in bone remodeling —caused by excess bone resorption or decreased bone formation relative to resorption— results in a loss of bone mass and quality, leading to osteoporosis and an increased risk of bone fractures [2].

Bone-resorbing osteoclasts are multi-nucleated cells derived from hematopoietic precursors of the monocyte/macrophage lineage, and their differentiation and function is controlled by cytokines such as receptor of activated nuclear factor kappa-B ligand (RANKL)[1] and its antagonist osteoprotegerin (OPG) [2]. Bone-forming osteoblasts differentiate from bone marrow stromal cells in response to Wnts and other growth factors; they secrete extracellular matrix proteins to produce osteoid, which becomes calcified to form mature bone [2]. During this process, some osteoblasts differentiate into osteocytes, which are completely embedded in mineralized matrix, but interconnected via cytoplasmic processes extending through micro-canaliculi. Osteocytes are long-lived and highly active cells, which sense mechanical stress and produce regulatory peptides, including sclerostin, a potent inhibitor of Wnt signaling [3]. Bone remodeling is controlled by systemic hormones, such as estrogens and parathyroid hormone, by locally-produced factors, such as RANKL/OPG and Wnts/sclerostin, and by small signaling molecules such as NO and prostaglandins [2,3]. Nitric oxide in bone has been the subject of several reviews in the past [4–7]; here we concentrate on NO functions in bone that are mediated via activation of soluble guanylyl cyclase (sGC) and production of cGMP.

2. Expression of NO synthases and regulation of NO synthesis in bone cells

All three NO synthase (NOS) forms have been identified in bone and in isolated osteoblasts and osteoclasts by RT-PCR and immunohistochemistry [8–15]. NOS−1 and −3 are constitutively expressed in primary osteoblasts from humans and rodents, with NOS activity stimulated by increases in intracellular calcium concentrations. Mechanical stimulation significantly increases NO production in osteoblasts and osteocyte-like cells, and this increase is blocked by calcium chelation, but the source(s) of mechanically-stimulated NO synthesis in osteoblastic cells remain controversial [11,16–19].

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[1] The following abbreviations were used: BFR, bone formation rate; BMD, bone mineral density; BMSC, bone marrow stromal cell; BV/TV, bone volume fraction; CNP, C-type natriuretic peptide, GC-B, guanylyl cyclase-B; GSK, glycogen synthase kinase; MAR, mineral apposition rate; NO, nitric oxide; NOS, NO synthase; OB, osteoblast; OC, osteoclast; OPG, osteoprotegerin; OVX, ovariectomy; PKG, cGMP-dependent protein kinase; RANKL, receptor of activated nuclear factor-KB ligand; sGC, soluble guanylyl cyclase.

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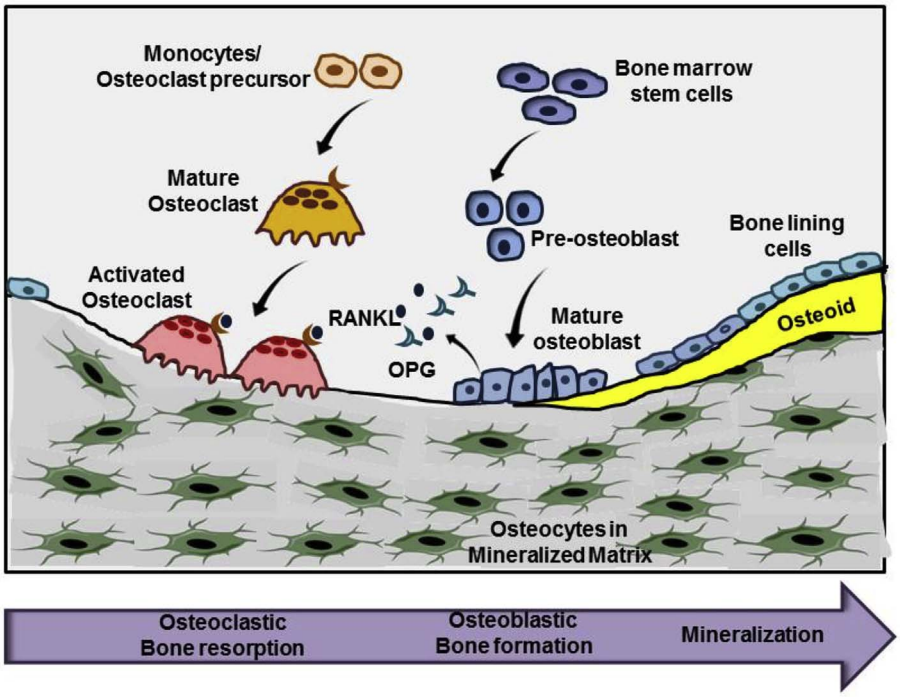

Fig. 1. Bone remodeling by osteoblasts and osteoclasts: Osteoclasts differentiate from hematopoietic precursors under the influence of RANKL, which is secreted by osteoblasts together with its antagonist OPG. Osteoclasts resorb calcified bone matrix and recruit osteoblasts to fill the defect with new bone. Osteoblasts differentiate from mesenchymal stem cells and secrete extracellular matrix to form osteoid. Osteoid calcifies to form mature bone and surrounds mature osteocytes.

Estrogens rapidly stimulate NOS-3 activation via a membrane-bound estrogen receptor; this requires an increase in intracellular calcium and Akt phosphorylation of NOS-3 in endothelial cells, with similar mechanisms operative in osteoblasts and osteocytes [20–24]. In addition, estrogen treatment increases NOS-3 mRNA expression over 24 h, likely via nuclear estrogen receptor binding to the NOS-3 promoter [25]. Thyroid hormone treatment of osteoblasts increases NO production via a membrane-bound thyroid receptor; thyroid hormone-induced NO synthesis is coupled to an increase in intracellular calcium concentrations and is abolished in NOS-3-deficient osteoblasts [26].

NOS-2 mRNA expression is regulated primarily at the transcriptional level, and is induced by inflammatory cytokines such as TNF-α, interferon-γ, and interleukin-1; NOS-2 expression in osteoblasts is also up-regulated by mechanical stimulation [9,10]. NOS-2 expression in differentiating osteoclasts is induced by RANKL, and exerts a negative feedback to restrict differentiation [13] (described below).

3. The bone phenotypes of NOS-deficient mice

NOS-1 knockout mice. NOS-1-deficient mice have an osteosclerotic phenotype with high bone mass, at least partly due to decreased osteoclastic bone resorption [27]. NOS-1-deficient mice demonstrate increased trabecular and cortical bone mineral density, and histomorphometry shows decreased osteoclast and osteoblast numbers, with reduced bone remodeling, reflected in low mineral apposition and bone formation rates [27]. Reduced osteoclast number and activity in NOS-1-deficient mice was confirmed in an *in vivo* model of inflammation-induced bone resorption [28]. Unexpectedly, more osteoclasts are formed from NOS-1-deficient compared to wild type bone marrow in the presence of RANKL and M-CSF *in vitro*, but the NOS-1-deficient osteoclasts are abnormally large and have reduced bone resorptive capacity [27,28] (Table 1). The high bone mass, low bone turnover phenotype of globally NOS-1-deficient mice may not be solely explained by defects of NOS-1 deficient bone cells, but indirect effects, e.g. hormonal changes due to NOS-1 deficiency in the nervous system, may contribute.

NOS-2 knockout mice. NOS-2-deficient mice do not have obvious bone abnormalities, with normal femur lengths, trabecular bone volume fraction, bone formation rate, and osteoclast surface [29]. However, NOS-2-deficient mice show altered responses to mechanical loading (discussed below, and Table 1).

NOS-3 knockout mice. Different strains of NOS-3-deficient mice have been generated with some differences in bone phenotypes, perhaps due to different genetic backgrounds [30–35]. NOS-3-deficient mice show defects in endochondral bone formation causing abnormal pre-natal and post-natal bone development, including fetal growth restriction, limb malformations, reduced longitudinal bone growth with hypocellular growth plates, and increased perinatal fatality [30–32]. Young (6–9 week old) NOS-3−/− mice demonstrate marked retardation in post-natal bone formation, not only reduced longitudinal growth —a function of chondroblast growth and differentiation in growth plates— but also reduced bone volumes associated with defects in osteoblast maturation and activity [32,33]. These NOS-3 −/− mice show markedly reduced osteoblast numbers and profound defects in bone formation and mineral apposition rates, with decreased trabecular bone volume and cortical thickness compared to wild type mice [32,33]. Osteoclasts appear to be unaffected by NOS-3 deficiency [32,33]. Bone densitometry scanning and micro-CT analysis show reduced femoral and spinal bone mineral density in 8 week-old NOS-3-deficient compared to wild type mice, with some investigators reporting that these abnormalities persisted at 20 weeks [33], whereas others found that the differences diminished and normalized by 12–18 weeks [32,35,36]. Altered responses of NOS-3-deficient mice to fluid shear stress or estrogen treatment after ovariectomy are discussed below (and Table 1).

In vitro, NOS-3-deficient osteoblasts proliferate slower and differentiate less well compared to wild type cells; both defects can be restored by the addition of exogenous NO [32]. NOS-3-deficient osteoblasts form less mineralized nodules, show reduced alkaline phosphatase activity, and express lower amounts of runx-2 (a master transcription factor for osteoblast lineage cells) and osteocalcin (an extracellular matrix protein important for mineralization) [32,33,36].

4. Cyclic GMP synthesis in bone cells

NO-stimulated cGMP synthesis and/or sGC expression have been documented in (pre)osteoblasts and osteoclasts [19,37–40]. Transcripts for the common, heme-containing β1 subunit of sGC are easily