Osteoarthritis and Cartilage 22 (2014) 1800–1807

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Review

Role of C-type natriuretic peptide signalling in maintaining cartilage and bone function

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ARTICLE INFO

Article history:
Received 19 March 2014
Accepted 22 July 2014

Keywords:
Osteoarthritis
Inflammation
C-type natriuretic peptide
Natriuretic peptide receptor
Cartilage homeostasis

SUMMARY

C-type natriuretic peptide (CNP) has been demonstrated in human and mouse models to play critical roles in cartilage homeostasis and endochondral bone formation. Indeed, targeted inactivation of the genes encoding CNP results in severe dwarfism and skeletal defects with a reduction in growth plate chondrocytes. Conversely, cartilage-specific overexpression of CNP was observed to rescue the phenotype of CNP deficient mice and significantly enhanced bone growth caused by growth plate expansion. *In vitro* studies reported that exogenous CNP influenced chondrocyte differentiation, proliferation and matrix synthesis with the response dependent on CNP concentration. The chondroprotective effects were shown to be mediated by natriuretic peptide receptor (Npr)2 and enhanced synthesis of cyclic guanosine-3′,5′-monophosphate (cGMP) production. Recent studies also showed certain homeostatic effects of CNP are mediated by the clearance inactivation receptor, Npr3, highlighting several mechanisms in maintaining tissue homeostasis. However, the CNP signalling systems are complex and influenced by multiple factors that will lead to altered signalling and tissue dysfunction. This review will discuss the differential role of CNP signalling in regulating cartilage and bone homeostasis and how the pathways are influenced by age, inflammation or sex. Evidence indicates that enhanced CNP signalling may prevent growth retardation and protect cartilage in patients with inflammatory joint disease.

Introduction

Articular joint function is known to be dependent on the homeostasis of multiple tissue types. Bones develop and grow into adulthood through the expansion and differentiation of chondrocytes at the cartilaginous growth plate through a process known as endochondral ossification. Initially, mesenchymal precursor cells condense in the centre and undergo differentiation into large chondrocytes that proliferate in a longitudinal manner characteristic of the growth plate[1]. The cells eventually stop proliferating and undergo terminal differentiation into hypertrophic chondrocytes that ultimately promote the vascularisation, mineralisation and recruitment of osteoclasts and osteoblasts which replace the calcified cartilage matrix with bone[2]. The process of hyaline cartilage formation that covers the articular surface leads to the deposition of extracellular matrix components and provides the structural properties that facilitate normal joint biomechanics. In turn, biomechanical signals promote beneficial anabolic activities in chondrocytes facilitating normal remodelling mechanisms and tissue homeostasis. However, in age-related diseases of the joint, the integrity of the extracellular matrix is lost, leading to disruption of cell function, abnormal signalling and reduced load-bearing function. Indeed, weakening of bone structure leads to osteoporosis (OP) and loss of cartilage integrity results in osteoarthritis (OA).

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http://dx.doi.org/10.1016/j.joca.2014.07.018
1063-4584/

It is well recognised that C-type natriuretic peptide (CNP) regulates cardiac and vascular function[3], promoting homeostatic effects involving hyperpolarisation and cell proliferation in a manner dependent on the cell type and concentration[4–7]. CNP is distinct from other natriuretic peptides, with emerging evidence for its ability to regulate cartilage and bone homeostasis. The development of transgenic mouse models that target the CNP signalling system has helped to define the importance of this peptide in cartilage and bone[8–18]. In clinical studies, dwarf patients with acromesomelic dysplasia, type Maroteaux (AMDM) were found to have autosomal recessive Npr2 functional deletions leading to stunted skeletal growth but no deficiency in CNP levels[19]. In addition, Npr2 functional halploinsufficiency that occurs in family members of AMDM patients were reported to have short stature[20]. This is in contrast to CNP overproduction which has been associated with bone overgrowth and skeletal deformations[21,22]. Human genome-wide association studies have demonstrated a link between CNP and height variation and circulating CNP levels predict height, with a close correlation to linear growth in healthy children[23,24]. Accordingly, plasma CNP levels are at a maximum at puberty, and gradually decline into adulthood[25]. The importance of the natriuretic peptide signalling pathway in the musculoskeletal system is therefore evident in both animals and humans. Consequently, there has been great interest in the biological effects of CNP and their influence on tissue homeostasis. In this review, we will discuss the role of CNP signalling in bone and cartilage tissues and how homeostatic functions may be influenced by age, inflammation or sex and lead to the onset and development of joint disease.

CNP/Npr2 signalling is critical for bone homeostasis

The skeletal defects observed in CNP and natriuretic peptide receptor (Npr) knockout mouse models are summarised in Table I and highlight the major phenotypes of CNP signalling in bone. CNP binds to two cell-surface receptors, Npr2 and Npr3, which are expressed in osteoblasts as well as chondrocytes. The effects of CNP are mediated *via* Npr2, which possesses guanylyl cyclase (GC) activity and leads to synthesis of cyclic guanosine-3′,5′-monophosphate (cGMP). Deletion of Npr2 mimics the skeletal defects associated with loss of CNP and defective Npr2 is responsible for dwarfism in patients with AMDM[19] and subsequently confirmed in animal models with loss of function mutation of the Npr2 gene in the cn/cn mouse[17]. In addition, carrier parents of children with AMDM were noted to be shorter than average[20,26]. Recent studies have also shown loss of function mutation in Npr2 leading to severe dwarfism, skeletal dysplasia and infertility in AMDM patients[8,17,27,28]. In animal studies, targeted disruption of genes encoding CNP showed severe dwarfism and early death as a result of impaired endochondral ossification characterised by a defective growth plate[9,12–14]. The inhibition in growth plate chondrocytes caused a 10% reduction in length in contrast to intramembranous bones which were of normal size[9]. In contrast, overstimulation of Npr2/GC causes skeletal overgrowth disorders[11,15,29,30]. Npr3 does not possess GC functionality, but acts as a clearance inactivation receptor and subjects natriuretic peptides to lysosomal degradation, thereby regulating Npr2 signalling[29]. Indeed, deletion of Npr3 in mice supports this model with significant overgrowth observed, most notably leading to distinctive arching of the back[29]. Furthermore, cartilage specific over-expression of CNP in growth plate chondrocytes rescued the phenotype of Nppc-deficient mice[11]. For example, no significant differences were observed in the naso-anal length between tg/Nppc$^{-/-}$ mice and Nppc$^{+/+}$ mice[9], clearly indicating that CNP is critical for bone formation.

The impact of the CNP signalling pathway *in vitro* appears to be dependent on the model system and celltype (Table II). Previous studies showed that CNP stimulated cell differentiation by increasing gene expression for alkaline phosphatase and osteocalcin and mineralisation of nodules in osteoblastic MC3T3-E1 cells and osteoblast-like cells from newborn rat calvaria[31–33]. These observations are consistent with the effects of CNP in primary cultures of fetal rat calvaria where the peptide induced osteoblast differentiation and showed synergy with factors such as osteogenic protein-1 (OP-1)[34]. The enhanced bone growth in mouse tibia cultured with CNP increased cGMP signalling through Npr2, and influenced the MEK (MAP/ERK kinase) Erk 1/2 kinase and p38 mitogen activated protein kinase (MAPK) cascades[35]. Recent studies reported differential effects of CNP on cell proliferation with the response dependent on the cell type[36,37]. In an earlier study, CNP was shown to activate osteoclast bone resorption activities in a cGMP-dependent manner in osteoclast containing mouse bone marrow cultures[38]. It is possible that the bone marrow cultures treated with CNP forced the undifferentiated stem cells in these cultures towards chondrogenesis *via* endochondral bone growth

Table I
In vivo evidence indicating a role for CNP/Npr signalling in the skeleton

Genotype	Phenotype	Reference
Nppc$^{-/-}$	- 10% loss of body weight at birth - 30–40% reduction of growth - No significant differences in organ/body weight - Stunted tail/bones - 50% birth rate - Reduced skull size and shortened jaws - Size and mortality rescued by systemic over-expression of CNP	[9–11]
Npr3$^{-/-}$	- Increased circulating ANP and ANP half-life - Normal birth rate, but 50% mortality before weening - Enhanced urine production and water intake - Hunched backs, dome-shaped skull, elongated tails and bones - Increased body length - Decreased weight	[29]
FGFR3$^{-/-}$	- Achondroplasia rescued by targeted cartilage-specific CNP expression and by systemic CNP over-expression	[62]
Prkg$^{-/-}$	- Dwarfism not rescued by targeted CNP over-expression, therefore linking CNP to cGKII signalling	[13]
Lbab/Lbab	- Long bone abnormality caused by a spontaneous mutation in CNP leading to reduced (30–100 fold) binding to Npr2 - Rescued by cartilage-specific expression of CNP	[14–16]
cn/cn	- Spontaneous missense mutation in Npr2 leading to dwarfism, short limbs and tail due to defective endochondral ossification - Notable lack of cGMP production in response to CNP	[17]
CNP^{col2a1TG} and K/BxN TCR	- Inflammatory arthritis model displaying growth delay, diminished chondrocyte proliferation and ECM synthesis - Symptoms rescued by cartilage-specific expression of CNP	[18]