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Adenosine and Bone Metabolism

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Abstract

Bone is a dynamic organ that undergoes continuous remodeling whilst maintaining a balance between bone formation and resorption. Osteoblasts, which synthesize and mineralize new bone, and osteoclasts, the cells that resorb bone, act in concert to maintain bone homeostasis. In recent years, there has been increasing appreciation of purinergic regulation of bone metabolism. Adenosine, released locally, mediates its physiologic and pharmacologic actions via interactions with G-protein coupled receptors and recent work has indicated that these receptors are involved in the regulation of osteoclast differentiation and function, as well as osteoblast differentiation and bone formation. Moreover, adenosine receptors also regulate chondrocyte and cartilage homeostasis. These recent findings underscore the potential therapeutic importance of adenosine receptors in regulating bone physiology and pathology.

Keywords

Adenosine; adenosine receptors; bone resorption; bone formation; chondrocytes

Purinergic receptors

Extracellular purines (adenosine, ATP and ADP) and pyrimidines (UDP and UTP) comprise a family of molecules that exert a variety of important physiological functions via the activation of cell-surface receptors termed purine receptors. Although the physiologic effects of adenosine and ATP have been recognized for over 80 years [1] purinergic receptors were first described in 1976 [2] and two subfamilies were identified; P1 or adenosine receptors and P2 or nucleotide receptors (Box 1). In this review we will primarily discuss the role of adenosine receptors in regulating bone metabolism.

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Conflicts of interest

AM holds patent adenosine A_{2A}R agonists to prevent prosthesis loosening (pending). BNC holds patents numbers 5,932,558; 6,020,321; 6,555,545; 7,795,427; adenosine A₁R and A_{2B}R antagonists to treat fatty liver (pending); adenosine A_{2A}R agonists to prevent prosthesis loosening (pending). BNC. is a consultant for Bristol-Myers Squibb, Novartis, CanFite Biopharmaceuticals, Cypress Laboratories, Regeneron (Westat, DSMB), Endocyte, Protalex, Allos, Inc., Savient, Gismo Therapeutics, Antares Pharmaceutical, Medivector, King Pharmaceutical, Celizome, Tap Pharmaceuticals, Prometheus Laboratories, Sepracor, Amgen, Combinatorx, Kyowa Hakka, Hoffman-LaRoche and Avidimer Therapeutics. BNC. has stocks in CanFite Biopharmaceuticals.

P1 or adenosine receptors

Adenosine is generated both intracellularly and extracellularly from the hydrolysis of adenine nucleotides (Figure 1), and acts locally to exert its extracellular physiologic and pharmacologic effects via activation of specific cell surface G protein coupled receptors (A_1 , A_{2A} , A_{2B} and A_3), proteins with unique pharmacological profile, tissue distribution and effector coupling [3]. As extracellular adenosine levels differ among tissues and in response to varying degrees of stress, the basal stimulation of each receptor by endogenous agonist varies [4]. Human adenosine receptors share 39–61% sequence homology among them and 11–8% homology with P2Y receptors, in the transmembrane domains (TMI-TMVII) [5]. Classically, adenosine receptors have been divided on the basis of their ability to inhibit (A_1 and A_3 receptor) or stimulate (A_{2A} and A_{2B} receptors) adenylyl cyclase activity [6–8]. The increase in cAMP levels following activation of $A_{2A}R$ and $A_{2B}R$, or the inhibition of cAMP generation by A_1R , leads to activation of other signaling systems such as activation of several types of K^+ or Ca^{2+} -channels, of phospholipase $C\beta$ and MAPK [3, 4, 9]. In some cell types like DDT₁MF-2, HEK93 and CHO cells, adenosine receptors activate mitogen-activated protein kinases (MAPKs), serine/threonine-specific kinases which further mediate downstream signaling events [8]. Interestingly, adenosine A_1 and A_2 receptors (A_1R and A_2R) have opposing functional effects [10]. For example, during inflammation, A_1R activation promotes multinucleated giant cell formation from peripheral blood monocytes, whereas $A_{2A}R$ activation inhibits this phenomenon [11]. A variety of selective adenosine receptor agonists and antagonists have been identified, based on structural similarities to agonists such as adenosine, or antagonists such as xanthines or caffeine and theophylline [4] (Table 1).

Bone homeostasis

Bones are rigid tissues that constitute the vertebrate endoskeleton that provides structure, support and protection, and facilitates movement. Bone also produces red and white blood cells, has a metabolic function with the storage of minerals, growth factors and fat, and acts as an endocrine organ [19]. Several cell types comprise bone such as osteoblasts and osteoclasts.

Osteoblasts

Osteoblasts are mononuclear cells derived from pluripotent mesenchymal stem cells that synthesize bone proteins, including sialoprotein and osteocalcin, and mineralize bone during bone formation and bone remodeling [20]. During the proliferation phase from precursor to mature osteoblast, these cells synthesize and secrete collagen type I [20, 21], which represents the majority (95%) of organic matrix proteins in bone [22]. Transcription factors responsible for the development of the osteoblast cell lineage including *runt*-related gene family such as core binding factor $\alpha 1$ (Cbfa1) and Runx2, as well as *c-fos* and *c-myc*. Osteoblast differentiation and growth is controlled by local and systemic factors such as fibroblast growth factor (FGF), insulin-like growth factor (IGF), bone morphogenic proteins (BMPs) and wnt/ β catenin [20].

Osteoclasts

Osteoclasts are the cells responsible for bone resorption. They are large, multinucleated cells derived from hematopoietic stem cells [23, 24], and are characterized by high expression of tartrate resistant acid phosphatase (TRAP) and cathepsin K. Osteoclasts are formed as a result of the stimulation by macrophage colony stimulating factor (M-CSF) and a complex network of regulatory factors that includes systemic hormones, locally produced cytokines and cell–cell and cell–matrix interactions [25, 26]. Receptor activator of nuclear factor

kappa-B ligand (RANKL) has been identified as the critical extracellular regulator of osteoclast differentiation and activation [27-31]. Binding of RANKL to its receptor, RANK, on the surface of osteoclast precursors, stimulates the activation of several intracellular pathways including nuclear factor-kappaB (NF- κ B), c-Fos, PLC γ , and nuclear factor of activated T cells c1 (NFATc1). Osteoblasts tightly control osteoclast differentiation by expressing RANKL on their surface and secreting this osteoclast stimulus. Further control is mediated by osteoblast-mediated secretion of osteoprotegerin, a decoy receptor for RANKL [32, 33].

Adenosine and Bone Metabolism

Within the field of purinergic signaling, the regulation of bone metabolism by extracellular nucleotides started as early as 1989 [34] and has been reviewed recently [35, 36]. However, little was known about the role of adenosine in controlling bone metabolism. Although adenine nucleotides are present at high concentration in the cell, their half-life is short and they are subsequently degraded [37]. However, under pathological conditions, such as hypoxia, stress or inflammation, adenosine concentration increases [4]. Indeed, adenosine and its receptors are involved in the (patho)physiologic regulation of different pathways in various tissues and organs (e.g. vasodilation airway tone) and adenosine receptor are targets of various drugs such as dipyridamole and the disease-modifying anti-rheumatic drug methotrexate. In addition, recent findings highlighting the significance of adenosine and its receptors in bone metabolism has opened new areas for exploration in understanding endogenous control of bone metabolism.

Role of adenosine and its receptors in osteoclast metabolism

Under physiological conditions, intracellular adenosine concentrations are relatively low ($<1\mu\text{M}$). Extracellular adenosine levels are regulated by both production and catabolism. Adenosine is produced extracellularly from adenine nucleotides by the action of a series of ectonucleotidases. The half-life of adenosine is short and a result of both facilitated cellular uptake, mediated by the transmembrane glycoprotein and member of the equilibrative nucleoside transporter family equilibrative nucleoside transporter 1 (ent1), and adenosine kinase-mediated rephosphorylation of adenosine, intracellularly [38]. In addition, adenosine is deaminated by adenosine deaminase to inosine. The recycling of adenosine and ATP is perturbed during conditions of metabolic stress, high cellular activity or cell death. These perturbations produce an increase in phosphatase activities, that together with an increase in diffusion, secondary transport of nucleotides and activation of ecto-5'-nucleotidases (CD73), result in an extracellular accumulation of adenosine and activation of adenosine receptors.

A₁R—In 1987, Fredholm et al demonstrated the presence of functional A₂ and P₂ receptors in mouse calvaria, but could not show presence of A₁-receptors [39]. Subsequent studies demonstrated the presence of all four adenosine receptors in murine bone marrow cell osteoclast precursors, splenocytes and in the monocytic cell line RAW264.7, and further demonstrated that these receptors were up-regulated during osteoclast differentiation [40]. Since this discovery, studies in bone cells (Figure 2), have revealed that A₁R is critical for osteoclast differentiation and function and that A₁R blockade (with the specific synthetic inhibitor DPCPX) or deletion (A₁ knockout mice) increases bone density and prevents ovariectomy-induced bone loss, in mice [40, 41]. This increased in bone density due to the blockade of A₁R results from suppression of RANKL-induced expression of the transcription factors NFATc1 and c-fos, the inhibition of RANKL-induced NF κ B activation. There is also a disruption in the RANKL-induced formation of a complex between TNF receptor associated factor 6 and the TAK1 kinase (TRAF6-TAK1), which is required during

osteoclastogenesis [42]. Moreover, He et al demonstrated that the A₁R is constitutively activated. Stimulation of the receptor by CPA does not directly affect osteoclastogenesis whereas the presence of the A₁R antagonists DPCPX or rollophylline, inhibit osteoclast formation [42]. These observations are consistent with the hypothesis that the A₁R antagonists act as inverse agonists, agents that diminish activity of a constitutively active receptor [43]. In contrast to these works, Pellegatti et al [44] reported modest expression of A₁R in both quiescent or MCSF/RANKL activated human monocytes, suggesting that A₁R does not play a role in osteoclastogenesis, while proposing that experimental disparities between studies may be due to species differences.

A_{2A}R—In contrast to A₁R, A_{2A}R activation opposes osteoclastogenesis. The A_{2A}R agonist, CGS21680, inhibits osteoclast differentiation and function and increases the percentage of immature osteoclast precursors, in part by decreasing IL-1 β and TNF α secretion [45]. Either antagonism or deletion of A_{2A}R abrogates stimulation of osteoclastogenesis *in vitro* and both dual-energy X-ray absorptiometry (DEXA) scanning and X-ray microtomography (microCT) examination demonstrated that mice deficient in A_{2A}R have a marked diminution of bone density, consistent with a marked increase in the number, and apparent function of, TRAP-positive osteoclasts [45]. Mediero, et al have reported that bone marrow-derived osteoclasts from normal and multiple myeloma patients are similarly affected by A_{2A}R [46], in contrast to the findings of Pellegatti and colleagues [44] who observed that A_{2A}R stimulation increases human osteoclast differentiation [44, 47]. The discrepancies among these groups may be explained by the different sources of osteoclast precursors used in the assays. Mediero et al performed their studies using bone marrow-derived osteoclast precursors, whereas the Pellegatti and Gartland groups used stimulated peripheral blood monocytes. This difference may also account for the variation in adenosine receptor expression and function resulting from blockade of the P2X₇ receptor.

The inhibitory effects of A_{2A}R on osteoclast differentiation may have therapeutic importance as well; as adenosine mediates the anti-inflammatory effects of the drug methotrexate [48] we speculate that the capacity of methotrexate to inhibit bone erosion in patients with rheumatoid arthritis (RA) may be mediated by adenosine stimulation of A_{2A}R. Consistent with this hypothesis is the finding by Mazzon et al that adenosine, acting via the A_{2A} receptor, reduces bone resorption in a collagen-induced mouse model of RA [49]. Mediero et al have reported another important *in vivo* role for A_{2A}R activation on osteoclasts. Hip and knee replacements are common, successful surgical interventions that are employed in an increasing number of individuals suffering from painful and degenerative diseases of the joints. In a model of wear particle-induced osteolysis, A_{2A}R agonists were shown to reduce bone pitting and loss, by diminishing inflammation and decreasing the number of osteoclasts [46].

A_{2B}R and A₃R—Little is known about the involvement of the other two adenosine receptors, A_{2B}R and A₃R, in osteoclast differentiation and bone resorption. In 1999, Xaus et al found that adenosine produced during inflammation was able to inhibit differentiation of murine bone marrow-derived macrophages in the presence of M-CSF, via the activation of the adenosine A_{2B} receptor (A_{2B}R) and subsequent production of cAMP [50]. These investigators [42] observed that the A_{2B}R agonist BAY606583 inhibits RANKL-induced osteoclast formation and expression of osteoclast markers, in murine cells. In contrast, Teramachi et al reported that adenosine abolished methotrexate-induced osteoclast inhibition by activation of A_{2B}R [51], although the effect on osteoclast differentiation was more likely due to an action of adenosine on osteoblast production of RANKL and osteoprotegerin following methotrexate treatment. An additional report documented the expression of A_{2B}R in human osteoclast precursors and differentiated [44]. However, the investigators did not observe any effect on osteoclast differentiation or function driven by A_{2B}R activation or

inhibition. Rath-Wolfson et al reported that IB-MECA, a potent and selective A₃R agonist, [52] ameliorates cartilage and bone destruction and decreases the number of osteoclasts, in a rat model of arthritis, although this effect is most likely secondary to diminished inflammation.

Collectively, these studies reveal a role for adenosine in osteoclast metabolism; the A₁R is constitutively activated and required for appropriate osteoclast differentiation and function whereas the activation of the A_{2A} receptor exerts the opposite effect that is inhibition of osteoclast function.

Role of adenosine and its receptors in osteoblast metabolism

In 1996 Shimeri et al reported that adenosine acts as a mitogen for the osteoblast cell line MC3T3-E1 [53], although the authors believed that since ATP had a stronger effect than adenosine, it was unlikely that this mitogenic action was caused by ectoenzyme-mediated ATP metabolites. It was later reported that methotrexate suppresses bone formation by inhibiting the differentiation of early osteoblastic cells in both MC3T3-E1 cells and rat bone marrow stromal cells [54], and that adenosine mediates many of the actions of methotrexate [55]. A subsequent study demonstrated the presence of extracellular adenosine and all four adenosine receptors in human osteoprogenitor cells [37]. Moreover, this work showed that there was a change in the secretion of two key modulators of osteoclastogenesis, osteoprotegerin, and the pro-inflammatory cytokine IL-6. Using the same cell line Russell et al reported in 2007 [56] that A_{2A}R regulates IL-6 secretion induced by lipopolysaccharide (LPS) treatment, suggesting a role for adenosine receptors in the control of inflammation, and potentially osteoclastogenesis and bone resorption. More recently, Costa et al investigated the presence and function of four adenosine receptors during differentiation of human bone marrow stromal cells from postmenopausal women *in vitro*; cells were cultured in osteogenic conditions where the adenosine that is generated from extracellular ATP catabolism exerts a powerful proliferative action [57]. The authors showed that selective agonists for A₁R, A_{2A}R, A_{2B}R and A₃R increase human primary osteoblast proliferation, and that selective receptor antagonists block proliferation (Figure 3).

However, additional controversies exist in relation to the presence of functional adenosine receptors and adenosine effects in primary calvarial cultures that need more exploration. Fredholm et al [39, 58] have reported the presence of A₂ receptors in osteoblast-like cells from neonatal mouse calvaria bones, whereas Jones and colleagues found no effect of adenosine on rat calvarial osteoblast cultures [58].

A₁R—Little is known about the role of the A₁R in osteoblast differentiation and function. As mention above, Fredholm et al demonstrated the presence of A₂ and P₂ receptors in osteoblast-like cells, but not the presence of A₁R [39]. Two recent papers [59, 60] show that A₁R is present in mouse mesenchymal stem cells and in 7F2 osteoblasts, although the stimulation of this receptor decreases alkaline phosphatase activity, a finding consistent with stimulation of adipocyte differentiation, as lipoprotein lipase activity is increased while alkaline phosphatase is decreased. Moreover, no morphologic changes in osteoblasts were observed in A₁R-knockout mice, and bone-labeling studies revealed no change in the bone formation rates [41].

A_{2A}R—As noted above, Russell et al [56] reported that adenosine inhibition of LPS-induced IL-6 secretion in an *in vitro* inflammatory model in the osteoblastic cell line MG-63 was due to the action of A_{2A}R on the cAMP/Protein Kinase A (PKA) pathway. Moreover, Katebi et al showed that adenosine, acting via A_{2A}R, plays a critical role in promoting the proliferation of mouse bone marrow-derived mesenchymal stem cells [61]. Finally, it was

recently reported that A_{2A}R is up-regulated in later osteoblast differentiation stages, and increases alkaline phosphatase activity [60]. Moreover, adenosine modulates cell viability in the osteoblast MC3T3 cell line in response to oxidative damage by hydrogen peroxide (H₂O₂) [62]. Both A₁R and A_{2A}R activation significantly increases cell viability and also protect against oxidative damage. When A₁R is blocked with the specific antagonist DPCPX, cell viability increases for up to 1 h after incubation, but the effect disappears when incubation is prolonged. In contrast, the A_{2A}R agonist CGS21680 increases cell number, when applied for 6 h at a low concentration, or at a high concentration and for a shorter period. Finally, the effects of A_{2A}R deletion on osteoblast function have not been reported.

A_{2B}R—Strong evidence implicates A_{2B}R in osteoblast differentiation and function. There is a decrease in osteoblast differentiation, osteoblast-related transcription factor expression and mineralized nodules in mesenchymal cells from A_{2B}R KO mice as compared to WT mice [63]. A_{2B}R is the predominant receptor in human osteoprogenitor cells [37], and Gharibi et al have also found that it is also the functionally predominant adenosine receptor in rat bone marrow-derived cells, and that its expression is transiently up-regulated at early stages of osteoblast differentiation. This transient overexpression of A_{2B}R induces the expression of the osteoblast-related genes Runx2 as well as expression and activity of alkaline phosphatase (ALP), a marker for osteoblast differentiation [60]. Costa et al recently reported that blockade of A_{2B}R with the specific antagonist PSB603 abrogated the increased osteoblast differentiation of human primary mesenchymal cells that were stimulated with the non-selective adenosine receptor agonist (NECA) [57]. This is in contrast to a prior report showing that this same agent does not affect proliferation in a human osteoprogenitor cell line, even though the concentrations used were similar in both studies. It was recently reported that A_{2B}R is important in osteoblast differentiation [64]. Indeed, when MC3T3-E1 cells overexpress the enzyme cluster of differentiation 73 (CD73) that converts AMP to adenosine, there is increased expression of osteocalcin and bone sialoprotein that is abrogated when A_{2B}R, but not the A_{2A}R, is blocked. These results are consistent with the hypothesis that CD73-generated adenosine activates A_{2B}R signaling to regulate osteoblast differentiation.. Subsequently Carroll et al [63] reported that osteoblast differentiation is mediated by a cAMP-related mechanism since activation of A_{2B}R or addition of the cAMP analog 8-bromoadenosine-3',5'-cyclic monophosphate (8-br-cAMP) enhances osteoblast differentiation in a parallel fashion. Moreover, when studied *in vivo* by microcomputed tomography analysis (μCT), long bones from adult mice lacking A_{2B}R had lower bone density than wild type animals, and suffered delayed fracture healing, with lower expression of osteoblast differentiation genes (Runx2 and Osterix). Finally, Gharibi et al [59] reported that although both A₁R and A_{2B}R are expressed in osteoblast precursors, A₁R induces adipocyte differentiation whereas A_{2B}R inhibits adipogenesis and stimulates osteoblast differentiation, suggesting that targeting these receptors may be useful in treatment strategies for diseases where an imbalance between osteoblasts and adipocytes exists. Thus, these studies are consistent with the hypothesis that A_{2B}R is a key modulator of skeletal repair and absence or blockade of A_{2B}R leads to delayed progression of bone development during fracture.

A₃R—Finally, with respect to A₃R, little is known. This receptor is expressed on osteoblasts and osteoblast precursors [37] and a selective agonist for A₃R increases human primary osteoblast proliferation, which is abrogated in the presence of the antagonist [57].

In summary, all four adenosine receptors are expressed by bone marrow stromal cells and differentiated osteoblasts. Although the A_{2B}R is dominant and is up-regulated during osteoblast differentiation, new evidence suggests the involvement of A₁R, A_{2A}R and A₃R in osteoblast differentiation.

Role of adenosine and its receptors in cartilage metabolism

When studying the role of purinergic signaling in cartilage metabolism, most of the attention has been on P2 receptors. Extracellular nucleotides have been shown to have diverse effects on chondrocyte function. Nonetheless, adenosine and its receptors have a role in regulating cartilage and chondrocyte function (Figure 4).

Adenosine metabolism—Children that suffer from a deficiency in the enzyme adenosine deaminase, a genetic mutation that leads to elevated adenosine levels in extracellular fluids, also suffer from severe combined immunodeficiency. These children exhibit marked changes in the chondro-osseous tissue of vertebrae and costochondral junction, have increased necrotic chondrocytes, and large amounts of cellular debris. All of these changes are ameliorated when patients received bone marrow transplants or multiple partial exchange transfusions [65]. This work suggested that adenosine might regulate cartilage biology. Along these lines, Tesch et al reported that equine articular chondrocytes [66] release adenosine in response to pro-inflammatory stimuli (LPS), and alteration of adenosine metabolism by an adenosine kinase inhibitor (5'-iodotubercidin, ITU) or the adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride (EHNA) increases accumulation of extracellular adenosine. This finding suggests that autocrine release of adenosine from chondrocytes may play a role in the cellular response to tissue damage in arthritic conditions.

A_{2A}R—Activation of A_{2A}R increases intracellular cAMP accumulation together with suppression of nitric oxide (NO) and these effects are enhanced by the adenosine deaminase inhibitor EHNA or by potentiating the cAMP response with the selective phosphodiesterase inhibitor rolipram [67]. Similarly, Petrov et al reported that inhibition of adenosine kinase by ITU attenuates IL-1 β -induced prostaglandin E₂ (PGE₂) release, and LPS-induced NO production in articular cartilage metabolism in metacarpophalangeal joints of young adult horses [68]. Moreover, Tesch et al revealed that endogenous adenosine levels regulate cartilage matrix homeostasis even in the absence of inflammation, in part due to the activation of cell surface adenosine receptors. Exposure to adenosine deaminase induces an increase in glycosaminoglycan release and production of total matrix metalloproteinase-3 (MMP-3), MMP-13, PGE₂, and NO, while blockade of A_{2A}R inhibits this effect [69].

Given that adenosine receptors regulate chondrocyte biology and cartilage matrix it was not surprising that adenosine and its receptors were reported to play a role in osteoarthritis in a murine model [70]. Furthermore, studies using polydeoxyribonucleotides (PDRN) a mixture of deoxyribonucleotide polymers with chain lengths ranging from 50 to 2,000 base pairs that have been shown to activate A_{2A}R, showed a significant amelioration in the clinical signs of osteoarthritis, reduction in cartilage expression and circulating levels of TNF α , and IL-6, and enhanced IL-10 expression. PDRN also reduced cytokine production from stimulated human chondrocytes [71]. In addition, all four adenosine receptors are expressed and similarly distributed in bovine chondrocytes and fibroblast-like synoviocytes. The A_{2A}R and A₃R are up-regulated with a subsequent increase in cAMP and cellular proliferation resulting in amelioration of joint disease [72]. Recent studies have confirmed and expanded the role of A_{2A}R in articular chondrocytes. Treatment of murine chondrocytes with the hyaluronan (HA)-blocking peptide Pep-1 and/or the specific A_{2A}R agonist, CGS21680, significantly reduced all the inflammatory molecules up-regulated by IL-1 β [73]. Moreover, treatment of primary murine chondrocyte cultures with the selective A_{2A}R agonist CV-1808 blocked up-regulation of the cell-surface glycoprotein CD44 and Toll-like receptor 4 (TLR4), and significantly reduced NF κ B activation and pro-inflammatory cytokine production [74]. The same work elucidated the roles of exchange protein activated by cAMP (EPAC) and PKA, showing a predominant EPAC involvement in mediation of the anti-

inflammatory activity of A_{2A}R. However, the same group has also reported that adenosine treatment increases intracellular cAMP and PKA activity and suggested, that hyaluronan (HA) fragments produced by degradation of native highly polymerized HA during inflammation, are partially responsible for the up-regulation of pro-inflammatory cytokines in chondrocytes, which is inhibited by adenosine receptor-stimulated activation of PKA [75].

It is clear that in response to inflammation, adenosine is released into the joint space and the activation of A_{2A}R ameliorates cartilage destruction, but more studies are needed for a better understanding of the role of adenosine in cartilage biology.

Concluding remarks

All four adenosine receptors are expressed by bone marrow cells, osteoclasts and osteoblasts, consistent with the hypothesis that adenosine and its receptors play a role in bone and cartilage homeostasis. The A₁R is critical for osteoclast differentiation and function, indeed agents that antagonize this receptor act as inverse agonists to inhibit osteoclast differentiation. In contrast, activation of the A_{2A}R inhibits osteoclastogenesis. In the case of osteoblast differentiation, it has been reported that A_{2A}R plays a critical role in promoting the proliferation of mouse bone marrow-derived mesenchymal stem cells, but the A_{2B}R is the functionally dominant adenosine receptor involved in osteoblast differentiation. Moreover, adenosine receptors regulate chondrocyte biology and cartilage matrix as the A_{2A}R and A₃R are up-regulated and their activation ameliorates joint disease. When studied *in vivo* all four adenosine receptors regulate bone formation in a manner consistent with their *in vitro* effects: A₁R blockade or deletion increases bone density; A_{2A}R activation inhibits inflammatory osteolysis whereas A_{2A}R blockade leads to excessive bone resorption and osteopenia; A_{2B}R deletion or blockade diminishes bone density. Future and ongoing investigations will help elucidate the intracellular pathways involved in adenosine receptor signaling that regulate bone metabolism. It will also be of interest to determine how well actions in human cells parallel those observed in mice and other species and how well these actions translate to new therapies for bone disease.

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Glossary box

Adenosine	purine nucleoside form of a molecule of adenine attached to a ribose sugar moiety via a β-N ₉ -glycosidic bond.
Adenosine receptors	class of purinergic receptors, G protein-coupled receptors with adenosine as endogenous ligand.
Bone formation (osteogenesis)	begins during prenatal development and persists throughout adulthood. There are two ways in which osteogenesis occurs: intramembranous ossification and endochondral ossification. Osteoblasts, used mainly in intramembranous ossification, meanwhile osteoclasts, used in are involved in remodeling of bone after formation.
Bone resorption	is the process by which osteoclasts break down bone.

M-CSF	Macrophage colony-stimulating factor, a secreted cytokine which influences hematopoietic stem cells to differentiate into macrophages or other related cell types.
Osteoarthritis	a group of mechanical abnormalities involving degradation of joints, including articular cartilage and subchondral bone. When bone surfaces become unprotected by cartilage, bone can be damage, resulting in a decreased movement secondary to pain, regional muscles atrophy and lax ligaments.
Osteoblasts	are mononuclear cells responsible for bone formation. They are specialized mesenchymal cells that express bone sialoprotein and osteocalcin. Osteoblasts produce a matrix of osteoid, which is composed mainly of Type I collagen.
Osteoclast	large, multinucleated cells derived from hematopoietic stem cells responsible for resorbing bone by removing the mineralized matrix and breaking up the organic bone. Osteoclasts are formed by the fusion of cells of the monocyte-macrophage cell line and are characterized by high expression of tartrate resistant acid phosphatase (TRAP) and cathepsin K.
Osteoprotegerin (OPG)	also known as osteoclastogenesis inhibitory factor (OCIF), or tumor necrosis factor receptor superfamily member 11B (TNFRSF11B), is a decoy protein that bind to RANK and inhibits osteoclast differentiation.
RANKL	Receptor activator of nuclear factor kappa-B ligand, also known as tumor necrosis factor ligand superfamily member 11 (TNFSF11), TNF-related activation-induced cytokine (TRANCE), osteoprotegerin ligand (OPGL), and osteoclast differentiation factor (ODF), is a critical for adequate bone metabolism. It is secreted by the osteoblasts and serves to activate osteoclasts by binding to its specific surface-bound membrane receptor RANK (receptor activator of nuclear factor kappa-B).
Rheumatoid arthritis	a chronic, systemic autoimmune inflammatory disorder that affect principally synovial joints. The pathogenesis of Rheumatoid Arthritis involves inflammation of the synovium with destruction of the articular cartilage and bone. Extra-articular manifestations of Rheumatoid Arthritis include inflammatory lesions of the lungs, pericardium, pleura and sclera.

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TEXT BOX 1: P2 or nucleotide receptors

There are two classes of P2 receptors: ligand-gated ion channels (P2X receptor, ionotropic receptors) or G protein coupled receptors (P2Y receptors, metabotropic receptors) [2, 5, 12]. P1 and P2 receptors have different pharmacological profiles and tissue distribution. Classically, P2X receptors are potently activated by stable ATP analogs such as α,β -methylene ATP (α,β meATP), and β,γ -meATP, whereas P2Y receptors are activated by 2-methylthio ATP (2MeSATP) [13, 14]. To date, seven mammalian P2X receptors, P2X₁₋₇, and eight P2Y receptors P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁, P2Y₁₂, P2Y₁₃ and P2Y₁₄ have been cloned, characterized pharmacologically and accepted as valid members of the P2 receptor family [15] (Table 2). P2X receptors are ATP-gated ion channels which mediate rapid (within 10 ms) and selective permeability to cations such as Na⁺, K⁺ and Ca²⁺ [16].

P2Y receptors are expressed on the surface of all cell types. Nevertheless, P2Y₁₁, P2Y₁₂, P2Y₁₃ and P2Y₁₄ receptors are known for their specific role in the stimulation of platelet aggregation and vascular tone [17]. P2Y receptors are primarily coupled by Gq to phospholipase C (PLC) with subsequent formation of inositol triphosphate (IP3) and diacylglycerol (DAG). P2Y₁₃ and P2Y₁₄ are also negatively coupled to adenylate cyclase through Gi, and P2Y₁ and P2Y₂ are also coupled to the RhoA / ROCK-I and ERK1/2 pathways [18].

TEXT BOX 1 TABLE I

P2 or Nucleotide receptors:

Receptor		Agonist (Rank potency)	Antagonist (rank potency)	Transduction mechanism	REF
P2X	P2X ₁	Bz-ATP >> 2-MeSATP ATP > α,β -MeATP >> ADP	NF449 > IP5I > TNP-ATP > RO 0437626 > NF279, NF023, RO1, MRS2159	Intrinsic cation channel (Ca ²⁺ and Na ⁺)	[2, 5, 12-14, 16, 17]
	P2X ₂	ATP ATP _γ S 2-MeSATP >> α,β -meATP	PSB-1011 > RB2, isoPPADS >PPADS > Suramin, NF770, NF778, aminoglycoside	Intrinsic ion channel (particularly Ca ²⁺)	[2, 5, 12-14, 16, 17]
	P2X ₃	Bz-ATP >> 2-MeSATP > ATP = α,β -MeATP	TNP-ATP, isoPPADS > A317491 > NF110 > PPADS, Ip5I, phenol red, RO4, RN-1838, Spinorphin, AF353	Intrinsic cation channel	[2, 5, 12-14, 16, 17, 76]
	P2X ₄	UDP=5Br-UDP >> UTP > 2-MeSADPBz-ATP = ATP	5-BDBD _ TNP-ATP, PPADS > BBG, Paroxetine, phenolphthalein,	Intrinsic ion channel (especially Ca ²⁺)	[2, 5, 12-14, 16, 17, 76]
	P2X ₅	ATP >> α,β -meATP > ADP	BBG > PPADS, Suramin	Intrinsic ion channel	[2, 5, 12-14, 16, 17, 76]

Receptor		Agonist (Rank potency)	Antagonist (rank potency)	Transduction mechanism	REF
	P2X ₆	ATP > 2-MeSATP > ADP		Intrinsic ion channel	[2, 5, 12-14, 16, 17] [76]
	P2X ₇	Bz-ATP > ATP > 2-MeSATP >> α,β -meATP	KN62, BBG, KN04, MRS2427, O- ATP, RN-6189, AZ10606120, A740003, A-438079, A-804598, GSK-1370319, Compound 31 (GSK), AZD-9056, CE-224535	Intrinsic cation channel and a large pore with prolonged activation	[2, 5, 12-14, 16, 17, 76]
P2Y	P2Y ₁	(N)-mc-2-MeSADP> 2-MeSADP> ADP=ADP β S >> ATP2-MeSATP> ADP> ATP2-MeSADP=2-MeSATP> ADP2-MeSATP> 2Cl-ATP> ATP	MRS2500 > MRS2279 > MRS2179, PIT, A3P5P	G _q /G ₁₁ PLC β activation	[2, 5, 12-14, 17, 18, 76]
	P2Y ₂	UTP=ATP> INS37217> Ap4A> ATP γ S> UTP ATP> ADP> 2-MeSATP UTP> ITP> ATP> UDP UTP=ATP> CTP> GTP UTP=ATP> Ap4A	AR-C126313 > Suramin> RB2, PSB-716, MRS2576	G _q /G ₁₁ and possibly G _i PLC β activation	[2, 5, 12-15, 17, 76]
	P2Y ₄	UTP> UTP γ S UTP=ATP=ITP=Ap4A UTP=ATP	ATP (human) > Reactive Blue 2 > Suramin, MRS2577, PPADS	G _q /G ₁₁ and possibly G _i PLC β activation	[2, 5, 12-14, 17, 76]
	P2Y ₆	UDP=5Br-UDP>> UTP> 2-MeSADP UDP> UTP> ADP> 2-MeSATP UDP> UTP> ADP> 2-MeSATP	MRS2578 > Reactive Blue 2, PPADS, MRS2567, MRS2575 (human)	G _q /G ₁₁ PLC β activation	[2, 5, 12-14, 17, 76]
	P2Y ₁₁	ARC67085 ATP γ S=BzATP> ATP> 2-MeSATP ADP β S=2-MeSADP 2-MeSATP>	NF157 > Suramin >	G _q /G ₁₁ and G _s PLC β activation	[2, 5, 12-14, 17,

Receptor		Agonist (Rank potency)	Antagonist (rank potency)	Transduction mechanism	REF
		ATP	RB2, 50-AMPS, NF340, AMP- a-5,		[76]
	P2Y ₁₂	2-MeSADP> ADP>> (N)-mc-2-MeSADP 2-MeSADP>> ADP, ATP 2-MeSADP> ADP> ATP 2-MeSADP> ADP> ADPβ S	AR- C69931MX> AZD6140, INS50589 > RB2 > 2-MeSAMP, AR-C66096, CT50547, PSB-0413, Carba- nucleosides, MRS2395, AR- C67085	G _i inhibition of adenylate cyclase	[2, 5, 12-14, 17, 76]
	P2Y ₁₃	2-MeSADP ADP> ADPβ S ADP> 2-MeSADP>> ATP ADP=2-MeSaDP=ADPβ S> ATP	AR-C69931MX > AR-C67085 > MRS2211, 2- MeSAMP	G _i	[2, 5, 12-14, 17, 76]
	P2Y ₁₄	UDP-glucosa> UDP-galactosa		G _{i/o}	[2, 5, 12-14, 17, 76]

Outstanding questions

- What intracellular pathways are activated by adenosine and its receptors to promote or inhibit osteoclast/osteoblast differentiation?
- Is there a translational role for targeting adenosine receptors in the therapy of bone resorption, arthritis and fracture healing?

Highlights

- Adenosine and its receptors are critical regulators of osteoclast differentiation.
- Adenosine A_{2B} receptor is critical for osteoblast differentiation but the other three adenosine receptors also play a major role in bone formation.
- Adenosine A_{2A} receptor may be an important regulator of cartilage loss.

Figure 1. Adenosine synthesis and receptor activation in the cell

Under physiological conditions, ATP is converted to adenosine via a series of dephosphorylation steps involving cytosolic ecto 5'-nucleotidases CD39 and CD73. Adenosine is converted to ATP via phosphorylation steps mediated by adenosine kinase (AK), and AMP kinase (AMPK). Both ATP and adenosine can be transported outside the cell via diffusion or active transport. Outside the cell, there is again ATP metabolic degradation to adenosine, which activates one of the four adenosine receptors (A₁R, A_{2A}R, A_{2B}R and A₃R). Once the receptors are activated, there is a positive (A_{2A}R, A_{2B}R) or negative (A₁R and A₃R) coupling to AC with the corresponding activation or inhibition of cAMP, that will differentially activate PKA, EPAC and several intracellular pathways.

Osteoclast precursor

Figure 2. Role of adenosine and its receptors in osteoclast metabolism

Under physiological conditions, intracellular adenosine concentrations are relatively low. **A₁R** is activated in osteoclast precursors and this produces osteoclast differentiation by RANKL-induced expression of the transcription factors NFATc1 and c-fos, induction of NFκB nuclear translocation and formation of the TRAF6-TAK1 complex. When adenosine levels increased due to inflammation for example, the other adenosine receptors are activated. Once **A_{2A}R** is activated, MCSF and RANKL secretion is inhibited, pro-inflammatory cytokine (IL-1β and TNFα) secretion is decreased while IL-10 expression is increased, leading to osteoclast differentiation inhibition. Little is known about how **A_{2B}R** and **A₃R** exerts inhibition of osteoclast differentiation.

Figure 3. Role of adenosine and its receptors in osteoblast metabolism

A₁R is present in mesenchymal stem cells and its activation produces the inhibition of osteoblast differentiation reflected in the decreased ALP activity observed. A_{2A}R is induced due to inflammation (IL-6), and in a cAMP/PKA-dependent mechanism it activates osteoblast differentiation. When A_{2B}R is activated, osteoblast differentiation and bone formation are increased by activating the expression of osteoblast-related genes Runx2 and ALP. A₃R is present in both osteoblast and osteoblast precursors and its activation increases osteoblast proliferation but little is known about the mechanisms involved.

Figure 4. Role of adenosine A_{2A}R in cartilage metabolism

When inflammation is produced in the articular cartilage (IL-6 and TNF α), adenosine is secreted and A_{2A}R is activated in chondrocytes, producing an increase in intracellular cAMP levels, together with a suppression of NO, and PGE₂ release, and enhanced IL-10 expression. This, in combination with activation of PKA and EPAC produce a decrease on inflammation and ameliorate the progression of illness and cartilage degeneration.

TABLE 1

P1 or Adenosine receptors:

Receptor	Agonist	Antagonist	Transduction mechanism	REF
A ₁	Adenosine N ⁶ -Cyclopentyladenosine (CPA)CCPA 2'-MeCCPA GR79236 S-ENVA CVT510	Caffeine Theophylline 8-Cyclopentyl-1,3 dimethylxanthine (CPX) 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) 8-Phenyl-1,3-dipropylxanthine PSB36 N-0840 MRS1754 WRC-0571	G _{i/o} diminish cAMP	[3-8]
A _{2A}	Adenosine ATL146e CGS21680 Regadenoson HE-NECA CVT3146	Caffeine Theophylline Istradefylline SCH58261 SCH442416 ZM241385 KF17837 KW6002	G _s increase cAMP	[3-8]
A _{2B}	Adenosine 5'-N-ethylcarboxamidoadenosine BAY606583 LUF5835 LUF5845 NECA (non selective)	Caffeine Theophylline CVT6883 MRS1706 MRS17541 PSB603 PSB0788 PSB1115 Enprofylline MRE2029	G _s increase cAMP	[3-8]
A ₃	Adenosine 2-(1-Hexynyl)-N-methyladenosine CF101 (IB-MECA) 2-Cl-IB-MECA CP532903 MRS3558 DBXRM VT160	Caffeine Theophylline MRS1191 MRS1220 MRS1334 MRS1523 MRS3777 MRE3008F20 PSB10 PSB11 VUF5574 L268605	G _{i/o} G _{q/11} decrease cAMP and increase IP ₃	[3-8]