Perspective Article

Bone anabolic therapy with selective prostaglandin analogs

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Abstract

Prostaglandin E₂ has been shown to increase bone mass in animals and humans but it also has considerable dose limiting systemic side effects. The molecular description of multiple seven transmembrane domain G protein coupled prostanoid receptors offered the opportunity to probe the skeletal effects of specific receptors using selective agonists. Bone effects have been reported with many of the prostanoid receptors, with most interest focused on the anabolic effects of EP2, EP4, and FP receptors. Current data suggests activity at the EP2 receptor stimulates formation, activity at the EP4 receptor stimulates resorption (and possibly formation), and activity at the FP receptor produces new trabeculae. However, caution must be exercised in extending the effects of prostanoids in isolated systems to systemic skeletal effects, since tissue level effects are the cumulative result of bone formation and bone resorption. Furthermore, species differences in receptor sequence and density confound extrapolation of effects from one model to another model. While these molecular targets increase our insight into how the skeleton can be affected pharmacologically, they still do not answer questions about the role of naturally occurring prostaglandins in skeletal health. This manuscript will review some of the recent advances in knowledge of the bone anabolic effects of selective prostanoid ligands.

Keywords: Prostanoid, Bone Anabolic, Osteoblast

The skeleton has been optimized through evolution to support two basic processes - 1) to provide a rigid framework (for locomotion and protection of internal organs), and 2) as a reservoir for mineral homeostasis. While the debate continues on whether bone strength or mineral homeostasis is most important for the health of the organism, there is little doubt that both are essential to host survival. The controls for mineral homeostasis (parathyroid hormone, calcitonin, and Vitamin D) have been identified through removal of specific organs such as the parathyroid and thyroid glands. However, little is known about the controls regulating mechanical homeostasis. Bone is known to respond locally to mechanical stress, such as the unilateral increase in forearm bone density in tennis players¹-². Conversely, bone that is no longer subject to mechanical stresses, as seen with denervation injury, is resorbed, although not completely³. Likewise, bone that is added during anabolic therapy is removed once therapy is discontinued⁴. Bones such as the skull and the femur respond differently to mechanical stress for maintenance of “normal” mass. This should not be surprising since evolutionary pressures for the skull integrity and femur integrity are likely to be quite different.

Osteocytes are well situated to sense the strain on bone, but they must communicate the need for increased bone mass through mediators to periosteal or endosteal osteoblasts. These mediators most likely diffuse rapidly and are inactivated in the systemic circulation. Both prostaglandins and nitric oxide are good candidates for mechanical signal transduction⁵ and may be useful targets for systemic bone anabolic therapy. In an osteocyte model system, cultured mouse calvarial cells exposed to pulsatile fluid flow increased their release of PGE₂, PGF₂α, and PGI₂, but with a different time course and to a different extent⁶. PGF₂α was increased in 5-10 minutes, PGE₂ release was increased by 10 minutes and continued over 60 minutes of treatment, and PGI₂ increased the slowest. The pulsed fluid flow also increased expression of prostaglandin G/H synthase-2 (PGHS-2) but not PGHS-1, which is responsible for production of prostaglandins, and may be important for long-term potentiation of the response⁶. These same mediators can also dilate local blood vessels causing an increase in blood flow⁷,⁸ or prevent osteoblast apoptosis⁹.

The prevention of apoptosis in osteoblasts or osteocytes by prostaglandins may also play a role in the tissue level
preservation or addition of bone mass. Peptides are also likely involved in mediation of prostanoid effects, as shown by the up regulation of IGF-1 in response to PGE₂ in cultured fetal rat calvaria. While these examples demonstrate local control of bone mass by prostaglandins, most studies have investigated the effects of systemically administered prostaglandins, since these studies are easier to perform and provide data on the potential use of prostanoids for osteoporosis treatment.

**Systemic effects of prostaglandins**

Systemic administration of prostaglandins has been known for many years to produce an increase in bone mass, bone volume, and bone strength. Prostaglandins have also been shown to increase bone mass in humans, both in neonates administered PGE₁ to maintain patency of the ductus arteriosus and in cases of infantile cortical hyperostosis. PGE₂ adds bone to the endosteal envelope by a modeling and remodeling dependent bone gain, often with formation of new trabeculae composed of woven bone at high doses. Periosteal formation occurs through a modeling dependent bone gain. In vitro cultures of periosteal osteoblasts from neonatal mouse calvaria demonstrate increased osteoblastic proliferation and bone formation in response to PGE₂. The timing and location of bone formation in response to PGE₂ has been summarized by Jee and Ma. It must be emphasized that the increase in bone mass is a cumulative result of a net positive bone balance, or when the rate of bone formation is greater than the rate of bone resorption. This point is especially important when predicting bone anabolic effects while studying the intracellular carboxyl terminus. Knockout mice now exist for all of these receptors. The reported phenotypes of these knockout mice have not included any gross skeletal abnormalities. This suggests either redundancy of pathways that locally control skeletal mass, or that the absence of one prostanoid receptor results in a compensatory increase in sensitivity of another prostanoid receptor.

Alternatively, mice may use different prostanoid receptors to control bone mass and may not predict the effects in other species. Based upon the receptor affinity profile, PGE₂ should stimulate all four EP receptors, resulting in an increase in both cAMP and calcium/inositol triphosphate in cells with all four receptors. This intracellular messenger profile is similar to the intracellular messenger profile of parathyroid hormone. The short half-life of PGE₂ also suggests the presence of a mechanism to potentiate the effect of PGE₂. The up-regulation of prostaglandin G/H synthase-2 and the production of additional prostaglandins have been suggested as a possible mechanism to prolong (potentiate) the effect of PGE₂. Potentiation also likely occurs through the production of peptides such as IGF-1.

**Selectivity of ligands**

Selective ligands are required to study specific receptor effects in vivo. The selectivity of numerous compounds have
been published for the mouse receptors and the human receptors\textsuperscript{27,31}. Doses at five to ten fold above the half maximal inhibitory concentration (IC\textsubscript{50}) of most naturally occurring prostanoids stimulates multiple receptors, confusing the measurement of specific effects of individual receptors\textsuperscript{27}. The lack of specificity of naturally occurring prostanoids may also be responsible for the biphasic or variable effects seen \textit{in vitro} since as the dose level of a non-specific prostanoid is increased, additional receptors are stimulated, often with opposing activities\textsuperscript{32}. For example, PGE\textsubscript{2} and PGF\textsubscript{2α} when combined with cortisol, both increased tritiated (\textsuperscript{3}H) thymidine incorporation. However, the selective FP agonist, fluprostenol was ineffective\textsuperscript{33}, suggesting the PGF\textsubscript{2α} effect was due to activity at an EP receptor rather the FP receptor. Conversely, since different prostanoid receptors use the same intracellular pathways, stimulation of two different receptors on a cell can result in similar results of an intracellular messenger (such as the EP2 and EP4 receptors, both of which elevate cAMP). This redundancy in the prostanoid pathways may also explain the lack of skeletal effects in prostanoid receptor knockout mice. Furthermore, induction of prostaglandin G/H synthase-2 to increase production of prostaglandins (auto amplification) further convolutes the study of specific agonists \textit{in vitro} or \textit{in vivo}\textsuperscript{30}.

While the most studied prostanoid is PGE\textsubscript{2}, the specific receptors mediating the effects on bone formation and resorption are not known. Specific agonists are known for the FP, TP, EP2 and IP receptors\textsuperscript{27}. New receptor specific compounds offer the potential to study the bone effects of additional individual receptors\textsuperscript{27}. Recent evidence points to the two relaxant receptors, EP2 and EP4, which both elevate cAMP, as playing a major role in mediating the effect of PGE\textsubscript{2}.

\textbf{EP2 effects}

The EP2 receptor is expressed in both long bones and calvaria of rats as shown by reverse transcriptase polymerase chain reaction (RT-PCR) and in situ hybridization\textsuperscript{34}. The mRNA levels were higher in growing rats compared to mature adults. Cultures of fetal rat calvaria respond to EP2 agonism with an elevation in cAMP resulting in replication and differentiation of osteoblasts. EP2 agonism in this system was proposed to be the major receptor responsible for PGE\textsubscript{2} anabolic activity\textsuperscript{33}. Recent work with new receptor selective EP2 analogs demonstrates an increase in bone volume and strength after local administration to the tibia of rats\textsuperscript{35,36}.

Resorption in mouse calvaria appears to be controlled by both EP2 and EP4 receptors\textsuperscript{37}. In highly purified osteoclasts from rabbits, PGE\textsubscript{2} inhibited bone resorption\textsuperscript{38}, which was attributed to EP4 activity, since it was the most abundantly expressed receptor and was inhibited by an EP4 agonist. However, an EP2 agonist (butaprost) also inhibited resorption in this rabbit \textit{in vitro} osteoclast model as well, suggesting a contribution of the EP2 receptor\textsuperscript{38}. In EP4 knockout mice, the EP2 agonist butaprost also increased resorption slightly\textsuperscript{39}. These effects on resorption by butaprost in these two models may be a result of either a role for the EP2 receptor in resorption or inadequate receptor selectivity of butaprost. In summary, activity at the EP2 receptor appears to stimulate osteoblast differentiation with less stimulation of osteoclast resorption, resulting in a net effect of a bone mass increase. \textit{In vivo} studies using local delivery\textsuperscript{35,36} have helped confirm these \textit{in vitro} findings. However, as stated earlier, species specific effects due to receptor distribution and sequence are likely responsible for subtle differences in net bone balance, and care should be taken in extrapolating effects between species.

\textbf{EP4 effects}

The EP4 receptor is expressed in osteoblastic cell lines (MC3T3, RCT-1, RCT-3, TRAB-11 and RP-1), in the tibia of young rats\textsuperscript{40} and in primary human osteoblasts\textsuperscript{41,42}. Additional \textit{in vitro} studies suggested that EP4 was the major prostanoid receptor subtype present in bone, was responsible for increasing cAMP, and up-regulates in response to ligand binding\textsuperscript{42}. 
In mouse calvarial cultures, resorption levels were increased with an EP4 agonist to a greater extent when compared to other EP receptor selective compounds. However, PGE₂ still produced the greatest increase in resorption. Additional evidence of the importance of the EP4 receptor in bone resorption comes from an impaired bone resorption response to PGE₂ in EP4 knockout mice. Cultured calvaria from EP4 knockout mice had no increase in resorptive activity when exposed to PGE₂. Calvaria from EP4 knockout mice still responded to dibutylryl cAMP with an increase in resorption. This data was taken to suggest that the EP4 receptor plays a major role in inflammation-induced bone resorption. Cell shape changes resulting in a more fibroblastic appearance. The EP1 receptor appears to contribute to the up regulation of PGE₂ production rates during PGE₂ administration (auto-amplification) based upon the increase in prostaglandin G/H synthase activity after in vitro addition of 17-phenyl trinor PGE₂. This auto-amplification may be crucial for prolonging bone growth in response to fractures or mechanical stress. No increase in resorption was seen in mouse calvarial cultures after treatment with an EP1 agonist. The EP4 receptor also appears to play a role in osteoclast formation by an EP4 antagonist. In an in vivo study, PGE₂ administered to young rats along with an EP4 antagonist greatly suppressed the bone formation response to PGE₂. The result was interpreted as evidence demonstrating a significant role for the EP4 receptor in stimulation of formation. However, this depressed formation could also represent an indirect result of depressed resorption which was not discussed in the publication. In summary, most of the literature suggests a role for the EP4 receptor in stimulation of resorption. However, there is indirect evidence that the EP4 receptor is also involved in bone formation. The relative balance of these effects in different species may lead to confusion on the bone anabolic potential of an EP4 agonist.

The EP4 receptor also appears to play a role in osteoclast activity associated with inflammation. In EP4 knockout mice, lipopolysaccharide administration caused no increase in osteoclastic activity as measured by urinary deoxyribonuclease. This increase in resorptive activity through the EP4 receptor appears to require both osteoblasts and osteoclasts, since in situ hybridization detected the EP4 receptor on osteoblasts, but not in multinucleated cells of mice. Furthermore, when a mixed culture from EP4 knockout mice was examined, IL-1α, TNFα, and bFGF were not elevated in response to PGE₂. This data was taken to suggest that the EP4 receptor plays a major role in inflammation-induced bone resorption (periodontal disease and osteomyelitis).

**EP1 effects**

The EP1 receptor is present on osteoblasts (Northern analysis in MC3T3 cells). The administration of an EP1 agonist, 17-phenyl trinor PGE₂, forced replication of cells rather than differentiation as measured by an increase in DNA synthesis and a decrease in alkaline phosphatase. The administration of 17-phenyl trinor PGE₂ also resulted in cell shape changes resulting in a more fibroblastic appearance. The EP1 receptor appears to contribute to the up regulation of PGE₂ production rates during PGE₂ administration (auto-amplification) based upon the increase in prostaglandin G/H synthase activity after in vitro addition of 17-phenyl trinor PGE₂. This auto-amplification may be crucial for prolonging bone growth in response to fractures or mechanical stress. No increase in resorption was seen in mouse calvarial cultures after treatment with an EP1 agonist.
which in vitro had only been shown to increase osteoblast proliferation and not promote osteoblast differentiation.

**Discussion**

In this review, we have summarized some of the current knowledge on the effect of prostaglandins on bone. The biphasic or variable effects seen with many studies of naturally occurring prostaglandins in vitro are most likely caused by stimulation of multiple receptors with compounds that lack sufficient selectivity. These occur less commonly in vivo, since there are limits to dose levels that are tested in vivo and stimulation of multiple receptors probably occurs to a lesser extent. This data also suggests that in vitro experiments with prostanoids should be interpreted with caution since most studies only examine formation or resorption and not both at the same time.

In the future as more selective compounds are described, we will achieve greater certainty of the role of each receptor and identify which may be the best target for a bone anabolic agent. The more selective compounds offer hope to identify a prostanoid that builds bone but does not suffer from numerous side effects such as increases in gastrointestinal motility and changes in blood pressure. Furthermore, since naturally occurring prostaglandins are rapidly inactivated or cleared from the circulation by a variety of mechanisms, such as oxidation of the 15-hydroxyl group in the lungs or removal of two carbons from the carboxyl terminus in the liver, new analogs may be designed with increased metabolic stability and increased half-lives.

While we have learned about many effects of specific receptors in vitro, we have limited in vivo knowledge of the activity of selective compounds. From our work with the FP selective prostanoids, there is a reminder that in vitro study of specific portions of the bone balance equation may help to understand, but will not always predict in vivo bone anabolic activity. A positive bone balance at the tissue level or the organism level is the result of a sustained greater rate of bone formation compared to bone resorption and must be measured in vivo with the full complement of mechanical, hormonal, and vascular components.

**References**


**Table 1.** Cloprostenol (0.3 to 100 μg/kg) and fluprostenol (3 to 1000 μg/kg) were administered at ½ log intervals for a total of six doses each (n=6/group). Cancellous bone volume was measured in the fourth lumbar vertebra and in the proximal tibial metaphysis (PTM). Bone mineral density (BMD) was measured in the mid- and distal femur by DXA.

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<th>Vertebral BV/TV (%)</th>
<th>PTM BV/TV (%)</th>
<th>Distal Femur-BMD (mg/cm²)</th>
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1985; 6(2):79-86.


