Drugs of the Future 2007, 32(9): 833-837

CCC: 0377-8282/2007
DOI: 10.1358/dof.2007.032.09.1124489

EP receptor agonists and other osteogenic therapies for bone repair

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Abstract

There is growing evidence that new molecules targeting either EP_2 or EP_4 receptors can enhance osteogenesis when injected locally, and hence may have potential application in bone repair. This could be of particular value in enhancing the healing of fractures and the bridging of surgical osteotomy gaps in children with growth defects. In addition, EP_4 agonists show promise in the prevention and restoration of ovariectomy-induced bone loss. Phase II and III trials with these compounds should allow their evaluation for use in humans, with the understanding that side effects due to the ubiquitous and overlapping distribution of EP receptors may pose a therapeutic challenge.

Introduction

Prostaglandins, particularly prostaglandin E_2 (PGE_2), are ubiquitously produced and have established functions in inflammation, embryo implantation, the induction of labor, vasodilatation and skeletal remodeling. These activities are mediated through the action of PGE_2 on one of four G-protein-coupled receptors: EP_1 through EP_4. EP_2 and EP_4 are coupled to $G_{s\alpha}$ and stimulate the production of cyclic AMP (cAMP) (1).

Clinically, the prostaglandin pathway has been the subject of drug discovery for arthritis and other inflammatory conditions. Small molecules have been developed that inhibit both isoforms of cyclooxygenase, COX-1 and COX-2. Two classes of COX inhibitors are currently used in practice: those that inhibit the COXs nonselectively, termed nonsteroidal antiinflammatory drugs (NSAIDs), and those that are selective for COX-2, termed COXBs (2). However, more recently, results from genetically manipulated mouse models that lack EP_2 and EP_4 receptors have shed new light on the function of prostaglandins in bone formation and bone resorption, paving the way for the future development of novel agonists for human use.

The function of prostaglandins in skeletal biology, particularly as it relates to bone repair, is well documented, and thus beyond the scope of this short review. Here we will focus on recent genetic and pharmacological studies that have clarified the osteogenic functions of the prostaglandin EP_2 and EP_4 receptors. We will examine the effects of synthetic small-molecule EP receptor agonists, such as CP-533536, in fracture healing and bone mass regulation. Finally, we will very briefly discuss other pharmacological and nonpharmacological interventions for fracture healing.

Genetic evidence for the role of EP receptors in bone remodeling

The EP_4-null mouse has been most well studied in terms of its skeletal phenotype. The mouse has evidence of reduced osteoblast differentiation and mineralization, and by 12 months of age develops significant osteopenia (3). The anabolic response to PGE_2 is substantially reduced both *in vivo* and in *ex vivo* cultures (4). In addition, there is a defect in osteoclast formation in response to PGE_2, likely due to an osteoblastic defect and a reduced production of receptor activator for NF-κB ligand (RANKL) (5). This defect is recapitulated in the EP_2-null mouse, suggesting that both EP_2 and EP_4 receptors modulate bone formation and resorption (6).

Further studies with EP_2- and EP_4-null mice demonstrate a defect in fracture healing. In the EP_4-null mouse in particular, there was evidence not only for an early slowing of fracture callus formation both in endochondral and membranous bone, but also for delayed cartilaginous

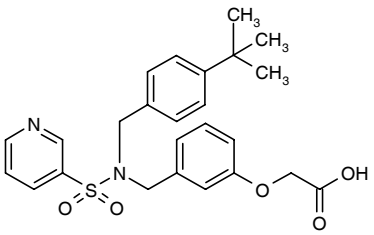
calcification in the more advanced stages (3). Together, loss-of-function studies support the premise that both EP_2 and EP_4 receptors are osteogenic, and therefore could serve as potential targets for a small-molecule agonist.

In contrast to the EP_2 and EP_4 receptors, the EP_3 receptor is thought to play a critical role in angiogenesis during wound healing. Thus, mice that lack the EP_3 receptor display impaired accumulation of $CD31^+$ and $VEGF^+$ (vascular endothlelial growth factor-positive) vascular endothelial cells (7). The effect was found to be transplantable: bone marrow cells from $EP_3^{-/-}$ mice induced a wound-healing defect in normal mice, indicating that vascular endothelial precursors were being mobilized from the bone marrow. This mouse has not been studied *vis-à-vis* a fracture healing phenotype, but it is likely that even in the absence of systemic osteoporosis, fracture healing may be inhibited due to reduced angiogenesis.

Pharmacological evidence for osteogenesis with EP_2 and EP_4 agonists

The first lines of evidence that PGE_2 is osteogenic *in vivo* came from several early studies. For example, PGE_2 was infused locally for 6 weeks on a plated osteotomy (8) and there was a dose-dependent stimulation of callus formation, which occurred mainly in the latter half of the 6-week infusion period. Because of the now-established role for both EP_2 and EP_4 receptors in osteogenesis, several receptor agonists have been developed for systemic and local use. These include the EP_2 agonist CP-533536 and the EP_4 agonists AE-1-329, ONO-4819 (rivenprost) and CP-734432. Here, we will discuss preclinical data that have demonstrated profound effects of these compounds in initiating new bone formation in a variety of experimental models.

CP-533536

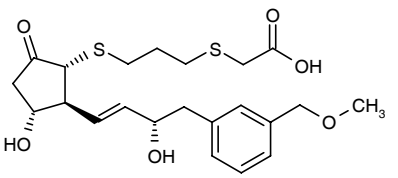


CP-533536 (**1**)

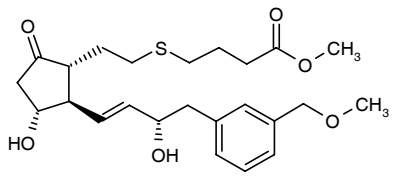
CP-533536 (**1**) is a highly selective nonprostanoid EP_2 agonist (9). Initial studies examined its osteogenic potential in male rats following its administration by intratibial injection. Profound new osteogenesis was evident locally as assessed by pQCT (peripheral quantitative computed tomography), bone histomorphometry and biomechanical testing. Subsequently, a single dose of 0.3 mg CP-533536 incorporated in a biodegradable polymer was implanted subperiosteally (10). New bone formation evident radiographically was coupled with histological evidence of trabecular bone without callus. The latter experiment suggested that new bone formation induced by CP-533536 in the uninjured periosteum involved the membranous rather than the endochondral bone formation pathway.

Having established the ability of CP-533536 to form new bone when injected either into bone marrow or subperiosteally, a standard fracture model was used to investigate its effect on bone repair. A single injection of CP-533536 into the matrix stimulated callus formation, followed by both intramembranous and endochondral ossification, and a fracture gap that was small (10). Biochemical testing revealed the restoration of bone strength. Likewise, when tested in the osteotomy model, a single dose of CP-533536 applied to the site of defect caused bone growth from both ends, resulting in the rebridging of the defect within 24 weeks (11).

AE-1-329 and ONO-4819



AE-1-329 (**2**)



ONO-4819 (**3**)

The EP_4 agonist AE-1-329 (**2**) was modified to create a long-lasting analogue ONO-4819 (rivenprost) (**3**). While the former was osteogenic when infused continuously, the latter compound only required thrice-daily injections. Systemic administration of ONO-4819 prevented bone loss and restored bone mass and strength in ovariectomized mice and attenuated bone loss following disuse. Higher doses of ONO-4819, however, caused hypotension and diarrhea, highlighting the inherent difficulty with such ubiquitously expressed targets in producing skeletal effects without undesirable side effects (5).

Two studies further examined the effect of ONO-4819 in bone repair. Twice-daily injection of ONO-4819 in a rat model of femoral diaphyseal drill-hole injury restored bone volume. However, RANKL and BMP-2 (bone morphogenetic protein-2) expression were also increased, suggesting that ONO-4819 accelerated both components of bone remodeling, formation and resorption (12). In a separate study, ONO-4819 contained within fibrin glue microspheres and implanted substernally corrected the

median sternotomy defect created in streptozotocin-treated diabetic rats (13). This model is relevant to sternotomy wound healing when diabetics undergo cardiac bypass surgery. It is possible that the incidence of sternal dehiscence could be attenuated by such implantations; however, the risk of systemic hypotension remains, even with small doses of locally released ONO-4819.

CP-734432

CP-734432 (structure not available) is yet another nonprostanoid EP_4 receptor agonist that triggered mineralized nodule formation *in vitro* in bone marrow cell cultures (14). When injected *in vivo,* the compound stimulated bone formation, and by doing so prevented and restored ovariectomy-induced bone loss at all sites –trabecular, periosteal and endocortical sites–, somewhat unique for a traditional anabolic agent.

Pharmacokinetcs and metabolism of CP-533536

The metabolism, excretion and pharmacokinetics of CP-533536 have been extensively studied (9). A summary of clinically relevant data obtained following i.v. injection of [^{14}C]-CP-533536 labeled at the benzylic carbon atom of the *tert*-butylphenyl ring is provided.

Radioactively labeled CP-533536 administered to rats was rapidly excreted, with > 90% recovery in a gender-independent manner within 24 h. Over 87% of the radioactivity appeared in feces, indicating that biliary excretion was the main route of elimination (9).

The pharmacokinetics of CP-533536 were different in male and female mice. While the elimination half-lives were similar in both sexes, the plasma clearance of total radioactivity and unchanged drug was 2-fold higher in male rats. In contrast, the steady-state volume of distribution was 2.6-fold higher in female rats (9).

Of note is that only 26% of the drug appears unchanged, with the remainder of the dose comprising phase I and II metabolites. These metabolites, characterized by LC/MS/MS (liquid chromatography/mass spectrometry/mass spectrometry), in combination with CID (collision-induced dissociation) mass spectrometry and a $TiCl_3$ reduction technique, are discussed in the original article by Johnson and Prakash (15). Of note also is that sex differences were observed in the nature of excreted metabolites, which was not unexpected, as such differences have been observed with other drugs (16). There is evidence that CP-533536 is metabolized by the CYP3A liver enzyme, which is expressed preferentially in the male liver (17).

Future of osteogenic therapies

Of the approximately 8 million fractures occurring in the U.S. every year, 10-20% display impaired or delayed healing (18). There are several new ways to promote the healing of such fractures. Most of these methods rely on the local application of bone-healing stimulants, the most commonly used being BMP-2 and -7, particularly BMP-7 (or osteogenic protein 1) (19). BMP-2 has been most stringently validated in humans in the BESST study (20). Such stimulants may be administered in a variety of vehicles, including collagen sponges, matrigels and microcapsules, or introduced using both viral and nonviral vectors (21).

Other peptides currently being tested include growth and differentiation factor-5 (GDF-5), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF) and VEGF (21). While a major concern with the use of BMPs is their propensity to cause ectopic calcification (22), results with TGF-β in animal models have been inconsistent (21). Results with VEGF, which enhances osteogenesis through accelerated angiogenesis, have been encouraging, particularly when administered using bacterial or viral vectors (23). In contrast, the effects of PDGF are of slow onset, and it has been difficult to maintain effective concentrations in the fracture microenvironment (24). Early clinical trials in patients with osseus defects in the periodontal space have nonetheless been encouraging (25).

In parallel, there is accumulating evidence that anabolic therapies, such as intermittently administered recombinant human parathyroid hormone (PTH), growth hormone (GH) and HMG-CoA reductase inhibitors (statins), can improve fracture healing by enhancing bone formation rates at the fracture site (21). Recombinant human PTH has shown promise in preclinical testing, and there is evidence for a sustained effect in promoting new bone formation (26). Clinical trials are under way to test the efficacy of PTH in humans. The role of statins in the treatment of osteoporosis remains debatable, although preliminary data on their effects in fracture models is compelling (27, 28).

The use of nonprostanoid EP_2 and EP_4 receptor agonists falls within the general category of small-molecule receptor mimics, which includes other molecules such as the thrombin-related peptide TP-508 (rusalatide acetate, Chrysalin®). TP-508 activates local platelets to release a number of cytokines, such as IL-12, IL-18, interferon gamma and bFGF. Despite a robust response in experimental models (29), clinical trials using TP-508 have been disappointing in their inability to demonstrate consistent differences in fracture healing between placebo and treated groups (21).

As discussed above, EP receptor agonists show enormous promise as they have direct effects in enhancing osteoblastic bone formation, but also induce angiogenesis and resorption (5, 12 13). Cardiovascular and gastrointestinal side effects are likely to limit their use at high doses, however. Local tendon degeneration has also been documented when PGE_2 was injected near the joint (30).

Various vectors and techniques for introducing drugs to the site of injury have been identified and are being tested. Of these, mesenchymal cells and muscle-derived stem cells have been extensively tested for their ability to carry genes of interest to the fracture site (31, 32). More

recently, vascular endothelial cells (33) and osteoblast precursors (34) have been isolated from the peripheral circulation with the ultimate purpose of redirecting genetically engineered cells to the site of injury. Drugs used to mobilize these precursors from their remote niches are also being considered (35).

The future thus holds significant promise for the utilization of new osteogenic molecules, including EP receptor agonists, and a myriad of vectors and new technologies that should allow the targeting of therapies to sites of bone injury, with minimal systemic side effects.

Acknowledgements

We gratefully acknowledge support from the National Institutes of Health (to MZ).

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