Title: METHODS FOR BONE TREATMENT BY MODULATING AN ARACHIDONIC ACID METABOLIC OR SIGNALING PATHWAY

Abstract: Methods for promoting osteogenesis to accelerate or enhance bone fracture healing, treat bone defects, and enhance bone formation are disclosed. The methods modulate an arachidonic acid metabolic or signaling pathway in general, and, in particular, utilize 5-lipoxygenase inhibitors. These molecules can be delivered alone or in combination with one or more agents that inhibit bone resorption, regulate calcium resorption from bone, enhance bone accumulation, enhance bone formation, induce bone formation, impair growth of microorganisms, reduce inflammation, and/or reduce pain.
TITLE
[0001] Methods for Bone Treatment by Modulating an Arachidonic Acid Metabolic or Signaling Pathway.

CROSS REFERENCE TO RELATED APPLICATIONS
[0002] This application claims the benefit of U.S. Provisional Application No. 60/709,838, filed August 18, 2005, the entire disclosure of which is hereby incorporated by reference in its entirety for all purposes.

FIELD OF INVENTION
[0003] The invention relates generally to accelerating or enhancing bone formation or fracture healing by modulating an arachidonic acid metabolic or signaling pathway, in particular by using inhibitors of 5-lipoxygenase activity.

BACKGROUND OF THE INVENTION
[0004] Bone fractures are a common traumatic injury. Approximately 8-10 million bone fractures are reported annually in the United States with more than 1 million of these requiring hospitalization. The estimated annual costs of treating these fractures exceeds 20 billion dollars. While this is already significant, these numbers are expected to increase due to the aging of the general population. Further, among military personnel, bone fractures are common training injuries. Bone fractures, typically located in the arms and legs, are also common battle wounds. Aside from traumatic injury, bone fractures also can be caused by disease. Osteoporosis is caused by a reduction in bone mineral density in mature bone and results in fractures after minimal trauma. The disease is widespread and has a tremendous economic impact. The most common fractures occur in the vertebrae, distal radius and hip. An estimated one-third of the female population over age 65 will have vertebral fractures, caused in part by osteoporosis. Moreover, hip fractures are likely to occur in about one in every three woman and one in every six men by extreme old age.

[0005] Fracture healing is a complex tissue regeneration process that involves cell migration, proliferation, apoptosis, and differentiation in response to growth factors, cytokines, other signaling molecules, and to the mechanical environment. The temporal order and magnitude of each cellular process must be controlled for optimal regeneration. The normal events of fracture healing are described below as occurring in 4 phases. In the initial phase, hematoma formation and localized tissue hypoxia are the initial cellular and molecular events of fracture healing. The second phase, called the early stage, is characterized by inflammation followed by rapid accumulation of cells at the fracture site. The presence of macrophages and neutrophils at the fracture site during inflammation precedes the rapid migration and
proliferation of mesenchymal cells at the fracture site. In the third, regenerative phase, endochondral ossification creates the new bone which bridges the fracture. At this point, the fracture callus has a well-defined morphology. Intramembranous ossification creates buttresses of periosteal bone at the callus periphery. Mesenchymal cells within the callus begin to differentiate into chondrocytes at the interface of the periosteal bone buttress. Each new chondrocyte develops as would be expected with matrix deposition followed by matrix calcification to produce calcified cartilage and then apoptosis. Channels are formed into the calcified cartilage starting at the periosteal bone buttresses. Osteoblasts migrate or differentiate on the surface of the calcified cartilage within these channels and begin depositing new bone. As chondrocyte differentiation proceeds from the periphery to the center of the callus (fracture site), channel formation, osteoblast differentiation, and new bone formation follows until the soft callus has been replaced with woven (immature) bone.

Angiogenesis during the regenerative phase is essential. The immature woven bone created during the regenerative phase is mechanically unsuited for normal weight-bearing. To compensate for the decreased mechanical properties of the woven bone, the fracture callus has a significantly larger diameter which provides for greater structural mechanical properties. In the final, remodeling phase, fracture callus diameter diminishes until the bone obtains its normal dimensions while maintaining the bones overall mechanical properties by enhancing material mechanical properties. This is accomplished by replacing the mechanically poor, woven bone with mechanically strong, lamellar (mature) bone. In successive rounds, osteoclasts resorb the woven bone and osteoblasts replace it with lamellar bone. Molecular mechanisms governing osteoclast formation and function occurs through the RANKL-RANK pathway and this pathway is activated during fracture healing.

Fractures are generally treated conservatively by closed reduction of the fracture and immobilization (casting) of the affected bone. In such cases, the bone heals through the endochondral ossification pathway described above. Adequate nutrition to include vitamin C, vitamin D, and calcium aids in healing. There has been no major advancement in the treatment of bone fractures since the mid 20th century when open reduction and internal fixation of fractures became commonplace. The promise of growth factor treatments to enhance fracture healing has not been realized yet.

Unfortunately, many fractures require surgical intervention to increase healing success and reduce the likelihood of complication. There is only one approved pharmacological enhancement for bone healing and that is treatment with recombinant bone morphogenetic protein, either BMP-2 or BMP-7 (OP-1). Use of these growth factors requires
surgery and due to expense and unknown potential side effects caused by the use of supraphysiological levels of growth factors, BMPs are used as a last-resort to heal recalcitrant fractures. Typical patient care also involves the administration of antibiotics, a narcotic, an NSAID, a COX-2 inhibitor or other pain killers during the healing process.

[0008] NSAIDs inhibit cyclooxygenase, thereby inhibiting the conversion of arachidonic acid into prostaglandins (PGD2, PGE2, PGF2α, PGI2, TXA2). Arachidonic acid is also a precursor for the leukotrienes (LTB4, LTC4, LTD4, LTE4), lipoxins (LXA4, LXB4), and 5-hydroxyeicosatetraenoic acid (5-HETE). The enzyme 5-lipoxygenase (5-LO) converts arachidonic acid to 5-hydroperoxyeicosatetraenoic acid (5-HpETE). This is the first step in the metabolic pathway which yields 5-HETE, the leukotrienes (LTs), and the lipoxins. Leukotrienes are also pro-inflammatory with the ability to attract neutrophils and cause capillary permeability. The arachidonic acid metabolic pathway is summarized in FIGURE 1.

[0009] Lipooxygenases are nonheme iron-containing enzymes found in plants and animals that catalyze the oxygenation of certain polyunsaturated fatty acids, such as lipids and lipoproteins. Several lipoxygenase enzymes are known, each having a characteristic oxidation action. Mammalian lipoxygenases are named by the position in arachidonic acid that is oxygenated. For example, the enzyme 5-lipoxygenase converts arachidonic acid to 5-hydroperoxyeicosatetraenoic acid (5-HpETE), while the enzyme 12-lipoxygenas converts arachidonic acid to 12-HpETE. The activity of 5-lipoxygenase requires a co-factor commonly called FLAP (five lipoxygenase activating protein). Leukotriene synthesis is reduced by drugs that inhibit FLAP (MK886) or mice lacking FLAP.

[0010] WO 95/30419 discloses 5-LO inhibitors reduce osteoclast activity. The suppression of osteoclast activity inhibits bone resorption and reduces bone loss in human pathological conditions. Bone resorption is an integral part of fracture healing because it is necessary to remodel the newly formed bone into stronger, more mature bone. The inhibition of bone resorption would be expected to impair the later stages of normal fracture healing.

Impair the later bone remodeling stage. The bisphosphonate effect on fracture healing reveals itself as persistence of a large fracture callus that contains mechanically immature, woven bone rather than mechanically mature, lamellar bone.

[0011] WO 03/066048 discloses that 12/15-lipoxygenase inhibitors can be used to prevent bone loss or increase bone mass. The publication describes data showing that bone mineral density is preserved in transgenic mouse that overexpress IL-4 and that were treated with a 15-LO inhibitor. The publication does not disclose that 15-LO inhibitors can aid fracture healing or the treatment of non-unions.

[0012] Traianedes, K., et al., 5-Lipoxygenase metabolites inhibit bone formation in vitro. Endocrinology, 139: 3178-3184 (1998) discloses the effects of LTB4, 5-HETE, and LTD4 (all products of 5-LO function) on the differentiation of fetal rat calvaria (osteoblast) cells. The data show that 5-HETE and LTB4 reduce bone nodule formation and alkaline phosphatase activity in vitro but that LTD4 had no effect. The results from an in vitro organ culture model showed that LTB4 or 5-HETE treatment prevented a BMP2 induced increase in mouse calvaria thickness. The publication, however, does not disclose the use of any 5-LO inhibitors, nor does it disclose that 5-LO inhibition would lead to the same effect in cultured osteoblasts or in organ cultures. Similarly, Ren and Dziak, Effects of leukotrienes on osteoblast cell proliferation. Calcified Tissue International 49: 197-201 (1991) discloses that LTB4 treatment reduces proliferation of primary rat calvaria (osteoblast) cultures in vitro, but that LTB4 can promote proliferation of established osteoblast cell lines (Saos-2 and G292) in vitro at higher concentration (0.3-1 micromolar). Ren and Dziak also disclose that LTC4 had no effect on the proliferation of primary rat osteoblast cells or Saos-2 cells but did promote proliferation of G292 cells. Further, Ren and Dziak disclose that treatment of Saos-2 cells with a 5-LO inhibitor (AA-861) had no effect on Saos-2 cell proliferation. The publication indicates that 5-LO inhibition should have no effect on osteogenesis.

[0013] Thus, it is readily apparent that compositions and methods for accelerating or enhancing bone formation or fracture healing would be highly desirable.

SUMMARY

[0014] The present invention provides methods of promoting osteogenesis by administering a compound that reduces a 5-lipoxygenase activity to treat a bone fracture, a bone defect or a condition treated by inducing bone formation.

[0015] In another aspect of the invention, the methods can further comprise an additional active agent such as a modulator of the activity of a cyclooxygenase. In one aspect the
activity of a cyclooxygenase-2 (COX-2) is increased. In another aspect, the activity of
cyclooxygenase-1 (COX-1) is reduced.

[0016] In one aspect, the methods use in vivo administration of a compound. In another
aspect, ex vivo administration of a compound is used.

[0017] In one aspect, the compound is a small molecule. In another aspect the compound is an
antisense compound. In another aspect, the compound is an RNAi compound.

[0018] These and other aspects of the present invention will become evident upon reference
to the following detailed description and attached figures. In addition, various references are
set forth herein which describe in more detail certain procedures or compositions, and are
therefore incorporated by reference in their entirety.

**BRIEF DESCRIPTION OF THE FIGURES**

[0019] Figure 1 summarizes an exemplary arachidonic acid metabolic or signaling pathway.

[0020] Figure 2 illustrates the modulation of arachidonic acid metabolism by altering
cyclooxygenase activity or lipoxygenase activity to accelerate or enhance bone formation.
FIGURE 2A represents the normal functioning of the pathway. FIGURE 2B shows that the
inhibition of COX-2 activity leads to excess leukotriene production which impairs bone
formation in fracture healing or other osteogenic processes. FIGURE 2C shows that the
inhibition of lipoxygenase activity leads to excess prostaglandin production which accelerates
or enhances bone formation in fracture repair or other osteogenic processes.

[0021] Figure 3 shows that serial x-rays of femur fractures made from a 5LO-/- mouse and a
normal mouse (C57BL/6). The x-rays show that osteogenesis, and therefore fracture healing
is accelerated in the 5LO-/- mouse.

[0022] Figure 4 illustrates mechanical testing data of fracture healing in wild-type (WT) and
5-LO knockout mice (5LO-KO or 5-LO-/-) 28 days and 84 days after the onset of the
fracture. Peak torque (FIGURE 4A), rigidity (FIGURE 4B), maximum shear stress (FIGURE
4C), and shear modulus (FIGURE 4D) were calculated from callus dimensions and the torque
to angular displacement curves.

[0023] Figure 5 illustrates histomorphometric data of fracture healing from wild-type (WT)
and 5-LO knockout mice (5-LOKO or 5-LO-/-) at 7, 10, 14, and 21 days after fracture. The
left panel shows the percent of fracture callus area that is newly formed bone (mineralized
tissue) and the right panel shows the percent of fracture callus area that is cartilage.

[0024] Figure 6 shows that fracture healing is dramatically impaired in COX-2 knock-out
mice and that the defect in healing occurs because of lack of osteogenesis (new bone
formation). FIGURE 6A shows data from x-rays and FIGURES 6B and 6C show the
histological samples of 14-day old femur fractures in mice lacking a functional COX-1 gene. FIGURE 6D shows data from x-rays and FIGURES 6E and 6F show the histological samples of 14-day old femur fractures in mice lacking a functional COX-2 gene.

[0025] Figure 7 illustrates that osteogenesis is accelerated in rats treated with 5-LO inhibitors, resulting in fractures healing faster than in untreated rats.

[0026] Figure 8 illustrates that osteogenesis is accelerated in rats treated with two different 5-LO inhibitors, resulting in fractures healing faster than in untreated rats. FIGURES 8A, 8B, and 8C show data from x-rays for vehicle control (8A), NDGA (8B), and AA-861 (8C). Figure 8D is a graph showing inhibition of 5-LO increases fracture callus peak torque.

**DETAILED DESCRIPTION OF THE INVENTION**


[0028] All publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety.

I. **DEFINITIONS**

[0029] In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

[0030] By "modulating an arachidonic acid metabolic or signaling pathway" is meant use of a drug or a compound which inhibits or promotes the activity or concentration of any enzyme or regulatory molecule involved in an arachidonic acid metabolism or signal pathway in a cell or animal. Preferably drug or a compound can be selected from a FLAP inhibitor such as BAYx 1005, MK-886, and MK-0591; a 5-Lipoxygenase inhibitor such as Zileuton, BAY-G576, RS-43,179, Wy-47,288, ABT-761, vitamin A, and BW A4C; leukotriene receptor antagonists such as zafirlukast, montelukast, pranlukast, ICI 204,219, MK-571, MK-679, ONO-RS-411, SK&F 104,353, and Wy-48,252; a leukotriene B4 receptor antagonists; a leukotriene C4 synthase inhibitors; a Leukotriene A4 hydrolase inhibitors; a non-steroidal
antiinflammatory drug (NSAID), a leukotriene receptor antagonists and leukotriene analogs, compounds modulating the formation and action of leukotrienes, compounds that affect cyclooxygenase activity, compounds that affect prostaglandin activity such as receptor agonists or antagonists, prostaglandin analogs, compounds that affect leukotriene activity such as receptor agonists or antagonists, and leukotriene analogs.

[0031] By “accelerated” is meant that osteogenesis occurs more rapidly and the time required for bone healing is reduced, or the bone heals more quickly in a treated subject as compared to an untreated subject or a control subject.

[0032] By “enhancing” is meant that the healed bone in the treated subject has improved characteristics compared to an untreated subject, or a control subject such as, for example, greater bone strength.

[0033] By “fracture healing” or “fracture repair” is meant that, in particular, promoting the healing of bone fractures and bone defects, and improving the mechanical stability of the healing fracture or site. Such bone fractures may be, for example, the common, traumatic (disabling and non-osteoporotic) fractures, the osteoporotic fractures due to osteoporosis or osteopenia of any etiology, fractures due to Paget's disease or fractures due to bone loss as a consequence of side effects of other drugs, e.g. in patients receiving high doses of corticosteroids, fractures arising from other congenital or acquired disease such as, e.g., osteogenesis imperfecta and breast cancer, surgical created fractures (osteotomies) used for example in bone lengthening and limb lengthening procedures, and treatment of bone fracture delayed unions or non-unions. The invention augments fracture healing following normal reduction and immobilization of the fracture using techniques common to one skilled in the art by accelerating and enhancing bone formation.

[0034] By “bone formation” is meant that the rate of bone formation in a subject treated according to the methods of the invention, such as, e.g., by receiving a 5-lipoxygenase inhibitor, is increased over the bone formation rate in a subject that is not given a 5-lipoxygenase inhibitor. Such enhanced bone formation is determined herein using, e.g., quantitative digitized morphometry, as well as by other markers of bone formation, as described above. Bone formation is meant to include the osteogenic process used for spine fusions and other joint or bone ankylosis application, bone formation into or around prosthetic devices, or bone formation to augment existing bones or replace missing bones or bone segments.

[0035] By “osteogenesis” is meant the production of bone that is associated with repair of a fractured bone, repair of a bone that has a defect caused by intentional or non-intentional
damage, or induction of bone formation used to fuse more than one bone or bone segment together. "Osteogenesis" is not meant to include bone formation associated with normal bone growth in adolescents. "Osteogenesis" also is not meant to include bone formation associated with normal bone homeostasis, which is often referred to as bone remodeling, in which bone is normally turned-over by a process whereby osteoclasts resorb bone and osteoblasts make new bone to replace that which has been resorbed.

[0036] By "bone defect" is meant damage to a bone such that a portion of the bone is removed or is otherwise missing. Such bone defects would include anomalous holes, gaps or openings created in the bone for purposes of a diagnostic or therapeutic procedure, loss of bone segments from trauma or disease, puncture wounds to the bone, and the like.

[0037] The term "modulating" refers to the effect of a modulator on an arachidonic acid metabolic or signaling pathway. A modulator can be, e.g., a polypeptide, nucleic acid, macromolecule, complex molecule, small molecule, compound, or the like (naturally occurring or non-naturally occurring) that is capable of causing modulation. Modulators can be evaluated for potential activity as inhibitors or activators (directly or indirectly) of a functional property, biological activity or process, or a combination thereof (e.g., agonist, partial agonist, partial antagonist, inverse agonist, antagonist, and the like), by inclusion in assays that measure the activity of an enzyme in the pathway.

[0038] The terms "effective amount" or "pharmacologically effective amount" refer to a sufficient amount of an agent to provide the desired biological result. That result can be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. For example, an "effective amount" for therapeutic uses is the amount of the composition comprising an active compound herein required to provide a clinically significant increase in osteogenesis and, thus, healing rates in fracture repair; reversal of cartilage defects or disorders; stimulation and/or augmentation of bone formation in fracture non-unions, delayed unions and distraction osteogenesis; increase and/or acceleration of bone growth into prosthetic devices; enhanced or accelerated bone formation in joint ankylosis, bone ankylosis, or spine fusions, bone formation to augment existing bone or replace missing bone or bone segments such as during autograft, allograft, or synthetic bone material incorporation, and repair of dental defects.

[0039] As used herein, the terms "treat" or "treatment" are used interchangeably and are meant to indicate administering one or more compounds in accordance with the methods of the invention to promote osteogenesis to obtain a desired therapeutic objective. The terms further include ameliorating existing bone or cartilage deficit symptoms, preventing
additional symptoms, ameliorating or preventing the underlying metabolic causes of symptoms, and/or encouraging bone growth.

[0040] As used herein, "small molecule" is meant to indicate a chemical compound having a molecular weight of less than about 500 daltons. Small molecules do not include biologic polymers such as polypeptides and polynucleotides.

[0041] By "pharmacologically acceptable" or "pharmacologically acceptable" is meant a material which is not biologically or otherwise undesirable, i.e., the material may be administered to an individual without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

[0042] By "physiological pH" or a "pH in the physiological range" is meant a pH in the range of approximately 7.2 to 8.0 inclusive, more typically in the range of approximately 7.2 to 7.6 inclusive.

[0043] As used herein, the term "subject" encompasses mammals. Examples of mammals include, but are not limited to, any member of the Mammalia class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. The term does not denote a particular age or gender.

[0044] The compounds of the present invention may be used to inhibit or reduce the activity of 5-lipoxygenase, 5-lipoxygenase and cyclooxygenase, and other enzymes and compounds in an arachadonic acid metabolic or signaling pathway. In this context, inhibition and reduction of the enzyme activity refers to a lower level of measured activity relative to a control experiment in which the enzyme, cell, or subject is not treated with the test compound. In particular embodiments, the inhibition or reduction in the measured activity is at least a 10% reduction or inhibition. One of skill in the art will appreciate that reduction or inhibition of the measured activity of at least 20%, 50%, 75%, 90% or 100% or any amount between 10% and 100%, may be preferred for particular applications. Inhibition of enzyme activity may be through any mechanism, including, by way of example, but not limitation, a reduction in the amount of enzyme present, a competitive or non-competitive inhibition of catalytic activity, an interference with an interaction between the enzyme and a co-factor or accessory protein, etc. In addition, the compounds of the present invention may be used to increase a COX-2 activity. In particular embodiments, the increase of enzyme activity refers to a higher level of measured activity relative to a control experiment in which the enzyme,
cell, or subject is not treated with the test compound. In particular embodiments, the increase in measured activity is at least a 10% increase. One of skill in the art will appreciate that an increase of the measured activity of at least 20%, 50%, 75%, 90% or 100% or any amount between 10% and 100% or beyond, may be preferred for particular applications. Increase of enzyme activity may be through any mechanism, including, by way of example but not limitation, an increase in the amount of enzyme present, or by increasing the enzyme’s turnover rate, or altering its substrate binding properties.

References to the enzymes 5-lipoxygenase (5-LO), COX-1, and COX-2 are intended to encompass the exemplary sequences referenced in Table 1, some of which are provided immediately following Table 1, as well as sequences at least 90% identical, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99% identical to the exemplary sequences as can be ascertained by one of ordinary skill using routine alignment algorithms such as e.g., BLAST. In addition, other mammalian homologues are encompassed. Such homologues are identified as such on the basis of e.g., sequence similarity, functional similarity, and by chromosome location. In addition to protein sequence, exemplary nucleic acid sequences are provided from which one of ordinary skill can readily obtain sequences of anti-sense and RNAi compounds useful for inhibiting the activity of the enzyme in accordance with the methods of the invention. Anti-sense compounds useful for practice of the invention are known in the art and can be obtained through commercial sources, as described in, e.g., Ding et al. (1999) BBRC Vol. 261, pp. 218-223 (incorporated by reference).

### Table 1 – Exemplary Sequences

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**Name:** prostaglandin-endoperoxide synthase 2; aka: cyclooxygenase-2, COX-2, PGHS-2

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**Name:** prostaglandin-endoperoxide synthase 1; aka: cyclooxygenase-1, COX-1, PGHS-1

1. Similarity between mRNA sequences.
2. Similarity between protein sequences.

**Human 5-Lipoxygenase mRNA Sequence** (GenBank RefSeq NM_000698) (SEQ ID NO: 1)

```
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Human FLAP mRNA Sequence (GenBank RefSeq NM_001629) (SEQ ID NO: 3)
Human FLAP Protein Sequence (GenBank RefSeq NM_001629) (SEQ ID NO: 4)

MDQRTVGWVULLAIVTLISVQNGFPAHKVEHESRTQNGRSPSTGTLAPERVYTANQCVDAYPTLAVLWSAG
LLCSQVPAAPAGMLYLFVRQYFVGYLGERSTQSTPGYIFGKRILFLFLMSVAGIFNYLIPPOSDFENYIKTI
STTISPLLLILP

Human COX-2 mRNA Sequence (GenBank RefSeq NM_00963) (SEQ ID NO: 5)

1 caattgtcat acgacttggca gtgagcgtca ggagcagtc caggaactcc tcagacgcgc
61 ctctttcagc tccacagccaa gcagccttca gcacgcacag cctaccgcgc gcgcgcgcgc
121 tgccgctcgc tcggcggtctc gcgcgcgcgc gtcgctgctg cgcgtccgcgc gcgcgcgcgc
181 atgcagaata cctctgtgct ctcacccccg gcagagactt gcagagactt gcagagactt
241 gattttgacca gcgttacccgc ccgacgatt gatagttgagc ccgacgatt gatagttgagc
301 cacccgaatt ttggaacaa ataagatatt ttctttacaag ccagcagcttc gtgtgccgcc
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541 gacgcgacgac gcgcgacgac gcgcgacgac gcgcgacgac gcgcgacgac gcgcgacgac
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661 atccccaggg ctctctctct ctctctctct ctctctctct ctctctctct ctctctctct
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841 gaaaaagtcg aatcgatcctt atgcgcgcgc gcgcgcgcgc gcgcgcgcgc gcgcgcgcgc
901 aggcagagct gcgtctcct ctcacagccc gcgcgcgcgc gcgcgcgcgc gcgcgcgcgc

961 aggtcttttg gtcgtggcct ggtctgatga tgtatgcac cacatctggct cgaggaacaca
1021 acagagatag cgtggtcgtt aacacgaggc atctcgaatg ggttgtagag cagttgtgcc
1081 agacaaacgg gctaatactg atagagaga gtttattaga tttgcagct gattatgtagc
1141 aacacttgag tggctatcac ttcmaaactg aatagccgcc gagaactatt ttcacaaacac
1201 atatccgctg cccaaacctgt ctcttcgcttg attaacaacc ctcctatcac tggctatcccc
1261 ttctgtcctga accatccctaaa atctcagacc agaataaaca ctaataccag tttatctaca
1321 acaccccttc attgctggaa cccatctgaa cccagctttt gtaattcatc accagcgaaci
1381 ttgctgcccc ggtggtcgtgt ggtagaaatg ttccacccgc agtcagaaaa gtcatacagg
1441 ctctctcttg cccagacagg cagatgaaat accagctttt taatacgta cgcacaagct
1501 ttgctgaaaa cccctcatgta cctttgtaag aacttacagg aagaaagaaa atgctgacag
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1621 aacaagctcg gccagatgcc atctttgtgtg aacacagtgt aagagtttga gcacattttc
1681 ctcgctaaag gtcagtcggt ttagtttatt gttctcctgc ttcacccgg agaagccactt
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1921 taactaaagaa acctggcgtct gaactcgtgaa agctctagta gcacatttat tttattatat
1981 gaccagtcct cttatcacta attactatat taatatatat taatatcctt tattttactt
2041 aacatctccct gtaacagagac tcagctcttc ttttttctga aaggtgctca tttttttgta
2101 gcttttaatt tcactactct aaagatttgg ctgttggtctg taagtttgga aacagttttt
2161 tatcctcttt tataaaccag agagaaatga tgttttgact ctttttactt gaatttcacac
2221 ttaattattta aacagaaag aacagatatt tgtaattacta aacactataca aagatggcca
2281 aatgctgtaa agtcttttaca tttctgatgt ttcacatgca ttcctcactg aagtttacag
2341 gtaaattgtg ttgcaataat taaatgttctt tgctgtctatc ctctctcagc ccaaaaaaac
2401 aggtactcct gctattttaa atgatatttt aaatttgaca ttacagctaa tttctgtcct
2461 acttttttaaa atgcagctaa ctttaataatatt attcatctaa aacatcctaa ggttagaactc
2521 acctgtgaaa gotttgttgga tttcttaaag ttattaaact tgtatcatata cccaaaaaag
2581 gctttgcttg gatttaaatct gtaaatacag atgtatattt tattttacta gttctgtaa
2641 tattttatat gttctgcctcc ttttttaccc aagagtataaa cttttttttt gttgactgtaa
2701 aacatctcccttt ttaatccatata cccaaatatt tattaagttg gtggagagcc tcagttttttaa
2761 tttcataataa aagatatttt tttgagatct ttcsgaatatt gttatatatg ctggtaacat
2821 gtaaactttta tcatcgcacaa aaggtcctacc ctttaaaataa ccataaaca aagaaagaaaac
2881 ccaatatttg ttcataatcc ggtttattat aacagctgaa actttttttt atcctgggtc
2941 actgcaagcccc tcagaagcttg aatattgctat gaggataatg aagttcaggt ctgtgcttga
3001 ataagcataa gtttttctcag atttttctgt tgcacagtta atttagcagct cccatataca
3061 tttcaaaaaagt acaagactgcc tcatatataa ctcctctaaat atctttatatt tcatcttctca
3121 cattaattttt actctactct tggagcaact tcagttgagg cattggaact aacgccctgtc
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3301 ttgagctctgg cttatattttt tcatctcgtgag tttggcagat cctcttacgcct ctcagctgcc
3361 aggactgcta tttagctcct tttaagaaga aaaaaaggg cctcttttttaa
Human COX-2 Protein Sequence (GenBank RefSeq NM_000963) (SEQ ID NO: 6)

HLRALLLLLCAVLASHTANPCCHSHCPQNRGVCMSVGFDQYKCDCRTTGFGYENCSTPBLTRIKLPLKPTNPVH
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VLKQEPHEWDOBQFQTSRLILGETIKVIEDTYHLQSYHFLKPKLDKLFFKNKPOVNINAAEBFNTLXHWP
LLPETFQXZDKXYQNYQPIFYNN3ILHPHTIQFVETRQIAGRAGRHNPAPAVQKQSA3ISPQMKYSQPN
EYRKRFMLKPYESFEELTGEKMSALEBYGALDADYPALLVBMKRPDAIFGTNMEVQAPFLSKGLNVI
CSPAYKPFSTFFGEGFQIIINTASIQSLICNNVGCFTSTFSVPDPELIKTVTINASSSGLDDINPTVLLKFR
STEL

Human COX-1 mRNA Sequence (GenBank RefSeq NM_000962) (SEQ ID NO: 7)

1 agggtgacagc tgtgagggagg aagggggggtg gagcgcggggg aagggtgagg agggtatgggg
61 cgccgagctcc gggccagtgt ccaggccccgc cccagggggtg ccagcgcccc gtcgccccgtg
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181 cctccgcccc gcctggtcgc cgtcgggggc cccagggggtg ccagcgcccc gtcgccccgtg
241 tgcctactcc gtcgcagcgc gttgcttctgc ttcctggttg ccctggtggtg cctggtggtg
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3061 ttccacaccca acagtgtgga aagctgttga taatctccat tccaaacccca aaggaagcacg
3121 ctcagagtggc tcagagtcaca cacctcagcgc agagtaggtgc cagaggtttg gctcctctcttg
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3241 gaatgtggtaa gtaaagcctc tcaagtgatac aatccagatcc cagatatagt gaatataaatga
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3421 acctaccattt cccgtttcgct tcccagtgact cccttcagtg cccttctgtagg
3481 aacccctcttg gttgtgcttct ccccctctcttg ctcctctcgc gaaataatcg cccttctgtagg
3541 ttcaggggca gacagccgctt cactcagcgtc tggagacccg tttctcaggg cccttctgtagg
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5041 ccaacccccca caatgttgtaa aatagatattc cccatctataaa gctatatata gttgggtgtg tgggtgctttct gggggtggtg
II. 5-LIPOXYGENASE INHIBITORS

[0045] The applicant has discovered that inhibiting the activity of 5-lipoxygenase promotes osteogenesis which can be used to accelerate and/or enhance the healing of a bone fracture, to treat a bone defect, or to treat by inducing bone formation. The applicant's discovery is based on his hypothesis that a potential mechanism by which loss of COX-2 function could inhibit fracture healing was by shunting arachidonic acid into the lipoxygenase pathway with consequent formation of abnormally high inhibitory 5-HETE, LTB4, or other 5-LO metabolite levels (FIGURE 2). During a normal inflammation response, such as a fracture, the synthesis of prostaglandins and leukotrienes is balanced (FIGURE 2A). Without being bound to a theory, the inventor theorizes that inhibiting COX-2 function shunts arachidonic acid into the lipoxygenase pathway to produce excess leukotrienes thereby impairing bone formation (FIGURE 2B). Conversely, by inhibiting 5-lipoxygenase activity, arachidonic acid is shunted into the cyclooxygenase pathway to produce excess prostaglandins that accelerate or enhance bone formation (FIGURE 2C).

[0046] To test this potential mechanism, fracture healing was assessed in 5-LO/- mice. The applicant found that loss of 5-LO function accelerates healing. Radiographic examination of fracture healing in age-matched mice in the C57BL/6 background showed that fracture bridging occurred by 2 weeks post-fracture in the 5-LO/- mice as compared to 3 weeks post-fracture in the normal mice (FIGURE 3). Further, callus remodeling was significantly accelerated, thus the 5-LO/- callus regains its initial structural and material properties much faster than in normal mice based upon torsional mechanical testing (FIGURE 4 and TABLE 2). Thus, loss of 5-LO function accelerates and enhances fracture healing and bone formation.

[0047] Histological examination of calcified samples supported the radiographic data. Plastic embedded, calcified sections of normal and 5-LO/- mouse fractures stained with Stevenel's blue and van Gieson's picrofuchsin show that after just 2 weeks of healing the fracture was bridged with calcified tissue in the 5-LO/- mice while the normal mouse (C57BL/6) still had a cartilaginous soft callus. Histomorphometric measurements of fracture
callus cartilage area showed that cartilage area peaked by day 7 post-fracture in 5-LO-/- mice and by day 10 post-fracture in normal mice (FIGURE 5 and TABLE 3). Measurement of new bone (calcified tissue) in the fracture callus showed that almost twice as much new bone in the 5-LO-/- after 7 days of healing and significantly more new bone at day 10 as well (FIGURE 5 and TABLE 2). These data show that a normal, albeit significantly accelerated, endochondral ossification pathway is used to heal the fracture in the 5-LO-/- mice. Experiments using younger and older 5-LO-/- mice and in different genetic backgrounds gave identical results: loss of 5-LO function results in accelerated bone regeneration.

[0048] The data from these experiments show that a 10 day fracture callus in 5-LO-/- mouse is equivalent to a 14 day callus in a normal mouse; that a 14 day 5-LO-/- callus is equivalent to a 21 day normal callus; and that a 1 month 5-LO-/- callus is equivalent to a 3 month normal callus (FIGURE 3). Thus, loss of 5-LO function accelerates and/or enhances the regenerative and remodeling phases of fracture healing.

[0049] In one aspect of the invention, compounds that inhibit 5-lipoxygenase activity accelerate and/or enhance healing of a bone fracture or prevent bone resorption or promote bone formation provide important benefits to efforts at treating human disease. Compounds that inhibit 5-lipoxygenase activity can be used, e.g., in a method for treating bone fracture due to trauma, or due to osteoporosis or osteoarthritis, in a method for treating Paget's disease, in a method for treating other conditions such as bone transplants and diseases associated with increased bone fracture, and in methods that require bone formation such as spine fusions, other bone and joint ankylosis procedures, bone or limb lengthening, augmentation of bone structure, incorporation of allograft, autograft, or synthetic bone material into bone defects, bone growth into or around prosthetic devices, and other similar procedures.

[0050] Several inhibitors of 5-lipoxygenase and their dosing are known which are useful for practicing the methods of the invention. A 5-lipoxygenase inhibitor can be 3-[1-(4-chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid (MK886) or derivatives thereof; 3-(1-(4-chlorobenzyl)-3-(1-butyl-thio)-5-(quinolin-2-yl-methoxy)-indol-2-y l)-2,2-dimethyl propanoic acid (MK-591) or derivatives thereof; nordihydroguaiaretic acid (NDGA) or derivatives thereof; 2-(12-hydroxydodeca-5,10-diynyl)-3,5,6-trimethyl-1,4-benzoquinone (AA861) or derivatives thereof; or (N-(1-benzo(b)thien-2-yl-ethyl)-N-hydroxyurea) (Zileuton) or derivatives thereof. Derivatives include, e.g., pharmaceutically acceptable salts, prodrugs, etc. which also are useful as 5-lipoxygenase inhibitors.
Derivatives of exemplary compounds are intended to be within the scope of the claimed invention.

kl)phenothiazine-1-carboxamide, Abbott A-65260, Abbott A-69412, Abbott-63162,
American Home Products AHR-5333, Bayer Bay-q-1531, Boehringer Ingelheim BI-L-357,
Boehringer Ingelheim BI-L-93BS, Boehringer Ingelheim BIL 226XX, Bristol-Myers Squibb
BMY-30094, carbazomycin B, Wellcome BW-B218C, Chauvin CBS-1114, Ciba-Geigy
CGS-21595, Ciba-Geigy CGS-22745, Ciba-Geigy CGS-23885, Ciba-Geigy CGS 24891,
cirsiliol, docebenone, Eisai E-5110, Eisai E-6080, enofelast, epocarbazolin-A, eprovafen,
evandamine, Fisons FPL 62064, Zeneca ICI-211965, Zeneca ICI-216800, Kyowa HakkoKF-
8940, Merck & Co L-651392, Merck & Co L-651896, Merck & Co L-652343, Merck & Co
L-656224, Merck & Co L-670630, Merck & Co L-674636, Lilly LY-233569, Merck & Co
MK-591, Merck & Co L-655240, nitrosoxacin-A, Ono ONO-5349, Ono ONO-LP-219, Ono
ONO-LP-269, Warner-Lambert PD-127443, Purdue Frederick PF-5901, Rhone-Poulenc
Rorer Rev-5367, Rhone-Poulenc Rorer RG-5901-A, Rhone-Poulenc Rorer RG-6866,
Roussel-Uclaf RU-46057, Searle SC-41661A, Searle SC-45662, Sandoz SDZ-210-610,
SmithKline Beecham SK&F-104351, SmithKline Beecham SK&F-104493, SmithKline
Beecham SK&F-105809, Synthelabo SL-81-0433, Teijin TEI-8005, Terumo TMK-777,
Terumo TMK-781, Terumo TMK-789, Terumo TMK-919, Terumo TMK-992, Teikoku
Hormone Tzi-41127, American Home Products WAY-120739, American Home Products
WAY-47288, American Home Products WAY-48252, American Home Products WAY-50295,
Yoshitomi Y-19432, 4-[[3-[4-(2-methyl-1H-imidazol-1-yl)phenylthio]]phenyl]-3,4,5,6-tetrahydro-2H-pyran-4-carboxamide, esculetin, phenidone and its derivatives, BL-I-239, 5,8,11-eicosatrynoic acid (ETI), 5,8,11,14-eicosatetraynoic acid (ETYA), cinnamyl-3,4-dihydroxy-alpha-cyanocinnamate, curcumin, esculetin, gossypol, caffeic acid, baicalein, 7,7-dimethylleicosadrenonic acid (DED), Ly311727, bromoeno lactone, methyl arachidonyl fluorophosphate, methyl y-linolenyl fluorophosphate, oleoxyethyl phosphorycholine, AACOCF3, n-(p-amylcinnamoyl) anthranilic acid, mepacrine, quinacrine, atabrine, parabromophenacylbromide, aristolochic acid, corticosteroids, Glaxo SmithKline 480848, Glaxo SmithKline 659032, Glaxo SmithKline 677116, BMS-181162, MJ33, and Millennium Pharmaceuticals MLN977.


[0054] In another aspect, the invention comprises a 5-LO inhibitor and a COX inhibitor and its use. Preferably, the COX inhibitor is a selective COX-1 inhibitor, i.e., that it inhibits the activity of COX-1 more than it inhibits the activity of COX-2. The use of a 5-LO inhibitor and a COX inhibitor is intended to embrace administration of each inhibitor in a sequential manner in a regimen that will provide beneficial effects of the drug combination, the co-administration of the inhibitors in a substantially simultaneous manner, such as in a single capsule having a fixed ratio of these active agents, or in multiple, separate capsules for each agent, as well as a single compound that inhibits both enzymes.
[0055] The COX inhibitor can be selected from the group consisting of celecoxib; rofecoxib; meloxicam; piroxicam; valdecoxib, parecoxib, etoricoxib, CS-502, JTE-522; L-745,337; FR122047; NS398; from non-selective NSAIDs that would include aspirin, ibuprofen, indomethacin CAY10404, diclofenac, ketoprofen, naproxen, ketorolac, phenylbutazone, tolfenamic acid, sulindac, and others, or from steroids or corticosteroids. Compounds which selectively inhibit cyclooxygenase-2 have been described in U.S. Pat. Nos. 5,380,738, 5,344,991, 5,393,790, 5,466,823, 5,434,178, 5,474,995, 5,510,368 and WO documents WO96/06840, WO96/03388, WO96/03387, WO95/15316, WO94/15932, WO94/27980, WO95/00501, WO94/13635, WO94/20480, and WO94/26731, and are otherwise known to those of skill in the art.

[0056] Selective COX-1 inhibitors are known in the art. The following is a list of preferred COX-1 selective NSAIDs: SC-560 [Smith et al., Proceedings of the National Academy of Sciences of the United States of America 95:13313-8 (1998)], FR122047 [Dohi et al., European Journal of Pharmacology 243:179-84 (1993)], Valeroyl salicylate, Aspirin. Aspirin is an irreversible cyclooxygenase inhibitor that is rapidly inactivated in vivo. While aspirin can inhibit COX-1 and COX-2, prior treatment with aspirin can inactivate all pre-existing COX-1 before or during expression of COX-2. Thus any new COX-2 that is expressed is active but all “older” COX-1 or COX-2 is inactivated.

[0057] The following is a list of NSAIDs that preferentially inhibit COX-1 versus COX-2: Dexketoprofene, Keterolac, Flurbiprofen, Suprofen. See also [Warner et al., Proceedings of the National Academy of Sciences of the United States of America 96:7563-8 (1999)].

[0058] In another embodiment, the invention comprises a 5-LO inhibitor and a COX-2 activator and its use. COX-2 activators also are known in the art. See [Tanabe and Tohrai, Prostaglandins & other Lipid Mediators 68-69:95-114 (20020) for review article concerning regulation of COX-2 gene expression and as a reference for those compounds or treatments listed below without a reference. Preferred COX-2 activators include ultrasound therapy [Sena et al., Ultrasound in Medicine & Biology 31:703-8 (2005)], pulsed electromagnetic fields (PEMF) [Lohmann et al., Journal of Orthopaedic Research 21:326-34 (2003)], BMP2 [Chikazu et al., Journal of Bone and Mineral Research 17:1430-40 (2002)], PDGF, FGF, and PTH and its analogs (PTHrP and teraparatide) [Maciel et al., Journal of Rheumatology 24:2429-35 (1997)]. Other COX-2 activators include Prostaglandins and prostaglandin receptor agonists [Rosch et al., Biochemical and Biophysical Research Communications 338:1171-8 (20050), PDGF (platelet derived growth factor), IL-1alpha (interleukin 1 alpha), IL-1beta, TNF-alpha (tumor necrosis factor alpha), FGF (fibroblast growth factor), TGF-beta
(transforming growth factor beta), TGF-alpha, EGF (epidermal growth factor), TPA (tetradecanoyl phorbol acetate),

[0059] In addition, the invention comprises a combination comprising a therapeutically-effective amount of a 5-lipoxygenase inhibitor and a cyclooxygenase-2 inhibitor, such as, e.g., licofelone, Dupont Dup 697, Taisho NS-398, meloxicam, flosulide, Glaxo SmithKline 406381, Glaxo SmithKline 644784, or tepoxatin.

[0060] The modulation of bone metabolism by the methods of the invention can be determined by examination of bone strength and mass after administration compared to a control subject. Such examination can be performed in situ by using imaging techniques (e.g., X-ray, nuclear magnetic resonance imaging, X-ray tomography, ultrasound, and sound conduction) or stress testing, or ex vivo by standard histological, radiographic, mechanical, or biochemical methods. Modulation of bone density and/or bone mass can be assessed by changes in one or more parameters such as bone mineral density, bone strength, trabecular number, bone size, and bone tissue connectivity. Several methods for determining bone mineral density (BMD) are known in the art. For example, BMD measurements may be done using, e.g., dual energy xray absorptiometry or quantitative computed tomography, and the like. Similarly, increased bone formation can be determined using methods well known in the art. For example, dynamic measurements of bone formation rate (BFR) can be performed on tetracycline labeled cancellous bone from the lumbar spine and distal femur metaphysis using quantitative digitized morphometry (Ling et al., Endocrinology 140: 5780-5788 (1999)). Alternatively, bone formation markers, such as alkaline phosphatase activity, serum collagen peptide levels, or serum osteocalcin levels can be assessed to indirectly determine whether increased bone formation has occurred (Looker et al., Osteoporosis International 11: 467-480 (2000)). Compounds that modulate an arachidonic acid metabolic or signaling pathway can be tested for their ability to accelerate or enhance fracture healing and/or bone formation, promote bone formation, and prevent bone loss. This can be tested in a variety of animal models well known to one skilled in the art such as animal fracture models, animal osteotomy models, animal skull trephine defect models, animal bone defect models, various animals segmental defect models and bone lengthening models, ovariectomy induced bone loss models, and the like. The utility of these animal models is well established and is supported by a wide range of different observations. For example, BMP2 studies in animals including rats demonstrated that BMP2 stimulates osteogenesis and BMP2 is now used clinically in humans for bone repair applications (tradename INFUSE). There are hundreds of papers about this in animals and tens of papers about humans; NSAIDs inhibit fracture

[0061] Modulation of bone metabolism by the methods of the invention can be determined in vitro by examining the proliferation, survival, and differentiation of osteoblasts and/or chondrocytes following treatment that alters arachidonic acid metabolism as compared to mock treated cells. Treatment of cells or organ explants such as newborn rodent calvaria or phalanges can be with compounds that inhibit 5-lipoxygenase activity, alter cyclooxygenase activity, affect leukotriene or prostaglandin receptor function, and the like as set forth in this application. Additional treatment methods can include use of antisense nucleic acids, interfering RNAs, other nucleic acid or proteins, and the like. Osteoblast or chondrocyte proliferation and survival can be measured by a number of techniques well known to one skilled in the arts such as cell counting, incorporation of radiolabeled thymidine or bromodeoxyuridine into replicating DNA, trypan blue exclusion, and terminal deoxynucleotidyl transferase end labeling of DNA within cells undergoing apoptosis. Differentiation of osteoblasts and/or chondrocytes can be measured by a number of techniques well known to one skilled in the arts and would include formation of mineralized nodules stained by the method of von Kossa or with alizarin red to ascertain osteoblast or chondrocyte culture mineralization, alcian blue staining of chondrocytes to measure elaboration of proteoglycan matrix, gene expression analyses to measure markers of osteoblast and chondrocyte differentiation such as Type I, Type II, and Type X collagen, osteocalcin, and aggrecan using protein or nucleic acid based assay methods, measurement of alkaline phosphatase activity, and measurement of RANKL, OPG, VEGF, bone morphogenetic protein, and other growth factors by quantitative methods such as enzyme-linked immuno assays (ELIA).

5-Lipoxygenase-activating Protein (FLAP)

[0062] FLAP is an 18-kD membrane-bound polypeptide which specifically binds arachidonic acid and activates 5-LO by acting as an arachidonic acid transfer protein. The FLAP gene spans greater than 31 kb and consists of five small exons and four large exons (GenBank
182657, Genbank M60470 for exon 1, Genbank M63259 for exon 2, Genbank M63260 for exon 3, Genbank M63261 for exon 4, and Genbank M63262 for exon 5).

[0063] The nuclear envelope is the intracellular site at which 5-LO and FLAP act to metabolize arachidonic acid, and ionophore activation of neutrophils and monocytes results in the translocation of 5-LO from a nonsecretionable location to the nuclear envelope. Inhibitors of FLAP function prevent translocation of 5-LO from cytosol to the membrane and inhibit 5-LO activation. Thus, FLAP inhibitors are anti-inflammatory drug candidates.

[0064] Leukotriene synthesis is reduced by drugs that inhibit FLAP (MK866) or in mice lacking FLAP. Thus, in one aspect of the invention, FLAP inhibitors such as BAYx 1005, MK-886, and MK-0591, are used in methods that modulate an arachidonic acid metabolic or signaling pathway thereby accelerating and/or enhancing fracture healing and bone formation.

Antisense Treatment

[0065] The term "antisense nucleic acid" is intended to refer to an oligonucleotide complementary to the base sequences of 5-LO or FLAP-encoding DNA and RNA or those that encode other proteins in an arachidonic acid metabolic or signaling pathway. Antisense oligonucleotides can be modified or unmodified RNA, DNA, or mixed polymer oligonucleotides, and, when introduced into a target cell, specifically bind to their target nucleic acid and interfere with transcription, RNA processing, transport and/or translation. Targeting double-stranded (ds) DNA with oligonucleotide leads to triple-helix formation; targeting RNA will lead to double-helix formation.

[0066] Antisense constructs can be designed to bind to the promoter and other control regions, exons, introns or even exon-intron boundaries of a gene. Antisense RNA constructs, or DNA encoding such antisense RNAs, can be employed to inhibit gene transcription or translation or both within a host cell, either in vitro or in vivo, such as within a host animal, including a human subject. Nucleic acid sequences comprising "complementary nucleotides" are those which are capable of base-pairing according to the standard Watson-Crick complementarity rules, where guanine pairs with cytosine (G:C) and adenine pairs with either thymine (A:T) in the case of DNA, or adenine pairs with uracil (A:U) in the case of RNA.

[0067] While all or part of the gene sequence may be employed in the context of antisense construction, preferably any sequence 17 bases long can be used to specify a unique target sequence. Although shorter oligomers are easier to make and increase in vivo accessibility, numerous other factors are involved in determining the specificity of hybridization. The antisense oligonucleotide is selected such that the binding affinity and sequence specificity to
its complementary target is sufficient for use as therapeutic agents. Thus, oligonucleotides of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or more base pairs can be used. One can readily determine whether a given antisense nucleic acid is effective at targeting of the corresponding host cell gene simply by testing the constructs in vitro to determine whether the endogenous gene’s function is affected or whether the expression of related genes having complementary sequences is affected.

Interfering RNA

[0068] Interfering RNA (RNAi) fragments, particularly double-stranded (ds) RNAi, can be used to modulate an arachidonic acid metabolism or signaling pathway. Small interfering RNA (siRNA) are typically 19-25 nucleotide-long RNA molecules that interfere with the expression of genes. Methods relating to the use of RNAi to silence genes in C. elegans, Drosophila, plants, and humans are known in the art (Fire et al., Nature 391: 806-811 (1998); Sharp, P. A. RNA interference 2001. Genes Dev. 15: 485-490 (2001); Tuschl, T. Chem. Biochem. 2: 239-245 (2001); WO0129058; and WO9932619).

[0069] The nucleotide sequence employed RNAi comprises sequences that are at least about 15 to 50 basepairs. The sequence can be a duplex, optionally with overhangs at the 5’-end and/or the 3’-end, where one strand of the duplex comprises a nucleic acid sequence of at least 15 contiguous bases having a nucleic acid sequence of a nucleic acid molecule within an arachidonic acid metabolic or signaling pathway. The length of each strand can be longer where desired, such as 19, 20, 21, 22, 23, 24, 25, or 30 nucleotides or up to the full length of any of those described herein. The single-stranded overhang can be, for example, 1, 2, 3, 4, 5, or 10 nucleotides long, and can be present at the 3’-end, the 5’end, or both the 3’-end and the 5’-end. Such fragments can be readily prepared by directly synthesizing the fragment by chemical synthesis, by application of nucleic acid amplification technology, or by introducing selected sequences into recombinant vectors for recombinant production.

[0070] In particular, the nucleotide sequences or RNAi can be oligonucleotides complementary to the base sequences of 5-LO or FLAP-encoding DNA and RNA or to the base sequences encoding other proteins in an arachidonic acid metabolism or signaling pathway. The oligonucleotides can be modified or unmodified RNA, DNA, or mixed polymer oligonucleotides, and, when introduced into a target cell, specifically bind to their target nucleic acid and interfere with transcription, RNA processing, transport and/or translation.

Other agents
[0071] In another aspect of the invention, an additional agent or drug may be administered to the subject. The additional agent can contain one or more active agents that effectively regulate calcium homeostasis, modulate chondrogenesis, modulate osteogenesis, modulate bone remodeling, regulate pain, regulate inflammation, or have antibiotic activity. The additional active agent can be, but is not limited to, an estrogen, an IGF, insulin, bone morphogenetic proteins and other growth factors, osteoprotegerin (OPG), a calcitonin, a bisphosphonate, vitamin D₃ or an analogue thereof, a statin, an adrogen, a fluoride salt, a parathyroid hormone or an analogue thereof, agents that enhance angiogenesis such as vascular endothelial growth factor (VEGF), agents that alter regulation of transcription of naturally occurring hormone regulators involved in bone metabolism, a vitamin, a mineral supplement, a nutritional supplement, and combinations thereof. The additional agent also may be an antibiotic such as gentamycin, ciprofloxacin, vancomycin, and/or others. This additional active agent can be administered to the subject prior to, concurrently with or subsequently to administration of the 5-lipoxygenase inhibitor of this invention. Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, p38 kinase inhibitors, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. Antibiotic compounds including but not limited to gentamicin, teicoplanin, tobramycin, and vancomycin, may also be combined in the composition of the invention.

III. PHARMACEUTICAL FORMULATIONS AND MODES OF ADMINISTRATION

[0072] The methods described herein use pharmaceutical compositions comprising the molecules described above, together with one or more pharmaceutically acceptable excipients or vehicles, and optionally other therapeutic and/or prophylactic ingredients. Such excipients include liquids such as water, saline, glycerol, polyethylene glycol, hyaluronic acid, ethanol, cyclodextrins, modified cyclodextrins (i.e., sulfobutyl ether cyclodextrins) etc. Suitable excipients for non-liquid formulations are also known to those of skill in the art. Pharmaceutically acceptable salts can be used in the compositions of the present invention and include, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients and salts is available in Remington's Pharmaceutical Sciences, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990).
Additionally, auxiliary substances, such as wetting or emulsifying agents, biological buffering substances, surfactants, and the like, may be present in such vehicles. A biological buffer can be virtually any solution which is pharmacologically acceptable and which provides the formulation with the desired pH, i.e., a pH in the physiologically acceptable range. Examples of buffer solutions include saline, phosphate buffered saline, Tris buffered saline, Hank's buffered saline, and the like.

Depending on the intended mode of administration, the pharmaceutical compositions may be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, suspensions, creams, ointments, lotions or the like, preferably in unit dosage form suitable for single administration of a precise dosage. The compositions will include an effective amount of the selected drug in combination with a pharmaceutically acceptable carrier and, in addition, may include other pharmaceutical agents, adjuvants, diluents, buffers, etc.

The invention includes a pharmaceutical composition comprising a compound of the present invention including isomers, racemic or non-racemic mixtures of isomers, or pharmaceutically acceptable salts or solvates thereof together with one or more pharmaceutically acceptable carriers, and optionally other therapeutic and/or prophylactic ingredients.

In general, compounds of this invention will be administered as pharmaceutical formulations including those suitable for oral (including buccal and sub-lingual), rectal, nasal, topical, pulmonary, vaginal or parenteral (including intramuscular, intraarterial, intrathecal, subcutaneous and intravenous) administration, in a form suitable for administration by inhalation or insufflation, or in a form suitable for administration at the bone formation site. The preferred manner of administration is oral or intravenous using a convenient daily dosage regimen which can be adjusted according to the degree of affliction.

Formulations for delivery at the bone formation site include adsorption onto or encapsulation within polylactide and/or polygalactide polymers, palmitic acid, alginate, plaster, calcium sulfate, calcium phosphate, mixtures of calcium sulfate and calcium phosphate, hydroxyapatite, collagen or other extracellular matrix material, bone wax (such as that from CP Medical, Inc., Ethicon, Inc., Unites States Surgical Corp., or Ceremed), Orthocon Bone Putty (a mixture of calcium stearate, vitamin E acetate, and alkylene oxide copolymer) or other materials or compounds that can be used for this purpose. Delivery can be accomplished by direct placement at the bone formation site or by deposition of the active
compound of the invention with or without a carrier onto the surface of prosthetic or surgically implanted devices.

[0078] A pharmaceutically or therapeutically effective amount of the composition is delivered to the subject. The precise effective amount varies from subject to subject and depends upon the species, age, the subject's size and health, the nature and extent of the condition being treated, recommendations of the treating physician, and the therapeutics or combination of therapeutics selected for administration. Thus, the effective amount for a given situation can be determined by routine experimentation. For purposes of the present invention, generally a therapeutic amount will be in the range of about 0.05 mg/kg to about 40 mg/kg body weight, more preferably about 0.5 mg/kg to about 20 mg/kg, in at least one dose. In larger mammals the indicated daily dosage can be from about 1 mg to 4,800 mg, one or more times per day, more preferably in the range of about 10 mg to 1,200 mg. The subject may be administered as many doses as is required to reduce and/or alleviate the signs, symptoms, or causes of the disorder in question, or bring about any other desired alteration of a biological system. One of ordinary skill in the art of treating such diseases will be able, without undue experimentation and in reliance upon personal knowledge and the disclosure of this application, to ascertain a therapeutically effective amount of the compounds of this invention for a given disease. When practicing the methods of the invention starting human doses may need to be estimated from rat dose data. Such estimation methods are well known in the art. See FDA publication “Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers” published July 2005 (Federal Register Document 5-14456) and available online at www.fda.gov/Cder/guidance/5541fnd.pdf. In general, the rat dose expressed as mg/kg should be divided by 6.2 to obtain an equivalent human dose.

[0079] When desired, formulations can be prepared with enteric coatings adapted for sustained or controlled release administration of the active ingredient.

[0080] The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.
IV. EXPERIMENTAL

[0081] Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

[0082] Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Example 1

5-LO Knock Out Mice

[0083] Knock out mice lacking 5-lipoxygenase (Alox5-/- or 5-LO-/-) were purchased from Jackson Laboratory, Bar Harbor, Maine. An impending femur fracture was stabilized with an intramedullary wire that was inserted retrograde into the femoral canal. A three-point bending device was used to make the fracture. Femur fracture healing was measured or assessed by histomorphometry, radiography, and torsional mechanic testing. The 5-LO-/- mice demonstrated statistically significant, quantitative acceleration and enhancement of fracture healing as compared to wild-type mice of identical genetic background and age (C57BL/6). Closed mid-diaphyseal fractures were made in 10-12 week old female mice. Fracture healing was assessed by x-rays (FIGURE 3) and quantitatively assessed by torsional mechanical testing 4 and 12 weeks after fracture (FIGURE 4 and TABLE 2). After 4 or 12 weeks of healing, the fractured femurs from 5LO-/- and wild type (WT) mice were excised and mechanically tested to failure in torsion using an MTS servohydraulic test machine and Interface 20 Nm torque load cell. Fractured femur dimensions were measured before and after testing. Peak torque, rigidity, maximum shear stress, and shear modulus were calculated from callus dimensions and the torque to angular displacement curves. All mechanical parameters were 50-120% higher after 4 weeks of healing in the 5-LO-/- as compared to the WT mice. Histomorphometric analysis of time-staged fracture specimens from normal and 5-LO-/- mice showed that cartilage area peaked early and to a greater extent in the 5-LO-/- mice (FIGURE 5 and TABLE 3). Further, significantly more new bone (mineralized tissue) was present in the 5-LO-/- fracture callus at 7 and 10 days after fracture. The data demonstrate that fracture healing is accelerated and enhanced in the 5LO-KO mice.

Table 2. Summary of fractured femur torsional mechanical testing data from 5-LO-/- and wild-type mice of identical genetic background and age at time of fracture (Fx).
Table 3. Summary of fracture callus histomorphometric analysis from 5-LO-/− and wild-type mice of identical genetic background and age at time of fracture.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sample Size</th>
<th>Days Post-Fx</th>
<th>Mean Percentages (Fracture/Contralateral) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peak Torque</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>9</td>
<td>28</td>
<td>85.3 ± 16.7</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>6</td>
<td>84</td>
<td>77.8 ± 20.1</td>
</tr>
<tr>
<td>Lox5-/−</td>
<td>8</td>
<td>28</td>
<td>128.5 ± 30.3</td>
</tr>
<tr>
<td>Lox5-/−</td>
<td>8</td>
<td>84</td>
<td>131.4 ± 26.0</td>
</tr>
</tbody>
</table>

[0084] The serial x-rays (FIGURE 3) show that fracture healing is accelerated in the 5LO-/− mice as compared to wild type mice (C57BL/6). More specifically, the 10 day old fracture from the 5LO-/− mouse appears to be at similar stage as the 14 day old fracture from the wild type mouse, the 14 day 5LO-/− fracture is similar to the 21 day wild type fracture, and the 1 month 5LO-/− fracture is similar to a 3 month old wild type fracture. The mechanical testing data show quantitatively that the structural and material properties of the 5-LO-/− fracture callus were statistically significantly better than the controls after 4 weeks of healing with a 50% increase in peak torque, a 75% increase in rigidity, a 75% increase in maximum shear stress, and over a 100% increase in shear modulus. Further, the 4 week mechanical testing parameters from the 5LO-/− mice were similar to those from the 12 week wild type mice, supporting the x-ray data of FIGURE 3 and demonstrating that fracture healing was accelerated and enhanced in the 5LO-/− mice. After 12 week of healing, the rigidity and shear modulus of the wild-type fracture callus had caught-up with the 5-LO-/− fracture callus.
Histomorphometric measurements of time-staged fracture callus specimens from the 5-LO/- and WT mice support the mechanical and radiographic observations (FIGURE 5 and TABLE 2). Callus cartilage area peaked by day 7 post-fracture in the 5-LO/- mice but not until day 10 in the WT mice. There was almost 4-times more cartilage present in the 5-LO/- callus at day 7 as compared to that from the WT mice. Concurrently, more new bone formation also occurred in the 5-LO/- mice with almost twice as much new bone (mineralized tissue) present at day 7 and 30% more new bone at day 10 as compared to the WT mice. The data is thus consistent with fracture healing occurring faster and producing more mechanically sound fracture callus with enhanced structural and material properties in the 5-LO/- mice than in normal mice.

Example 2

COX-2 Knockout Mice

Fracture healing was assayed in mice with a targeted deletion of the COX-2 gene. Closed, mid-diaphyseal femur fractures were made in the right hindlimb of COX-2 knockout, COX-1 knockout, and wild type mice (not shown). Fracture healing was assessed by x-rays and histology (FIGURE 6), and by mechanical testing (not shown). The data show that fracture healing was dramatically impaired in the COX-2 knockout mice, but not the COX-1 knockout or wild type mice. X-rays after 14 days of healing show a large mineralized fracture callus in the COX-1 knockout mouse (FIGURE 6) with little or no evident mineralized callus in the COX-2 knockout mouse. Histological examination confirmed the x-ray findings in that the COX-2 knockout callus had a significant amount of cartilage but no new bone was evident. Torsional mechanical testing data shows that fracture callus structural and material properties are statistically significantly worse than COX-1 knockout or wild type mice. When combined with the experimental results of example 1, example 3, and example 4 this demonstrates how arachidonic acid metabolic or signaling can be manipulated according to the methods of the invention to affect bone formation.

Example 3

Treatment of Rats with a 5-Lipoxygenase Inhibitor

fracture was stabilized with an intramedullary stainless steel pin. Beginning 4 hours after fracture the rats were treated with 30 mg/kg of NDGA (nordihydroguaiaretic acid) in 1% methylcellulose (5-lipoxygenase inhibitor treatment group) or with carrier only (1% methylcellulose). The day after surgery and continuing until day 14 post-fracture, experimental rats were treated with 2 doses of NDGA (30 mg/kg), the first dose between 8-10AM and then again with another NDGA dose 8-10 hours later. Control rats were treated similarly but with carrier only (1% methylcellulose). Three weeks after fracture, the rats were sacrificed, the fractured femurs were harvested, and high resolution radiographs were made of the fractured femurs using a Packard Faxitron and Kodak MinR2000 mammography film. Two representative radiographs are shown in FIGURE 7 for each treatment group: control and 5-lipoxygenase (5-LO) inhibitor treated.

[0087] The radiographs show that after 3 weeks the fractured femurs of the 5-LO inhibitor treated rats were bridged with new bone. In contrast, a well-formed, mineralized fracture callus has formed in the control rats but the fracture site had not yet bridged with new bone. In rat C, the fracture is bridged with new bone on the medial (top) and lateral (bottom) sides of the fracture callus. In rat D, the fracture is bridged with new bone on the lateral side (bottom) and shows indications of new bone bridging on the medial side. No new bone bridging is evident in the control rats (rats A and B). The data thus demonstrates that 5-LO inhibitor therapy can accelerate the fracture healing process in young, normal rats.

**Example 4**

**Treatment of Rats with 5-Lipoxygenase Inhibitors**

[0088] Sprague-Dawley rats (3 months old) underwent a standard closed femur fracture procedure as described in the art (Simon et al. Journal of Bone and Mineral Research, 17(6): 963-976 (2002); Bonnarens and Einhorn, Production of a standard closed fracture in laboratory animal bone. Journal of Orthopaedic Research, 2: 97-101 (1984)). The impending fracture was stabilized with an intramedullary stainless steel pin. Beginning 4 hours after fracture the rats were treated with vehicle (1% methylcellulose) or inhibitors of 5-LO suspended in 1% methylcellulose. Inhibitor A (NDGA) was administered at 30 mg/kg and Inhibitor B (AA-861) was administered at 5 mg/kg. The day after surgery and continuing until day 21 post-fracture, experimental rats were treated with 2 doses of inhibitor (either A or B), the first dose between 8-10AM and then again with another dose 8-10 hours later. Control rats were treated similarly but with carrier only (1% methylcellulose). Three weeks
after fracture the rats were anesthetized and high resolution radiographs were made of the fractured femurs using a Packard Faxitron and Kodak MinR2000 mammography film (FIGURES 8A, 8B, and 8C). Five weeks after fracture the rats were sacrificed, femurs resected, and assayed for structural mechanical properties by torsional mechanical testing (FIGURE 8D).

[0089] The radiographs showed that after 3 weeks of healing, the fractures appeared bridged in the 5-LO inhibitor treated rats but not in the vehicle treated rat.

[0090] Torsional mechanical testing was used to measure the peak torque sustained by each femur after 5 weeks of healing. The data show that the femurs from the Inhibitor A (NDGA) treated rats and from the Inhibitor B treated rats had 22% and 53% greater peak torque than vehicle treated rats (FIGURE 8D). In addition, all of the femurs from the Inhibitor A or B treated rats failed as boney unions while 13% (2 of 15) of the femurs from the vehicle treated rats failed as non-unions with no apparent bone bridging.

[0091] These experimental observations demonstrate that 5-LO inhibition therapy can accelerate (faster bone bridging) and enhance (better mechanical properties) fracture healing.

**Example 5**

**Ex Vivo Treatment Methods Using Small Molecule Compounds, RNAi, and Antisense Compounds**

[0092] Methods to promote ex vivo osteogenesis are used, e.g., to aid in healing of recalcitrant bone fractures, segmental defects caused by traumatic injuries or pathological resection of bone segments, or for joint arthrodesis. In these instances, precursor bone cells are isolated from a subject or from a suitable donor and are cultured ex vivo using standard methods. The cells are grown in or seeded into an appropriate scaffold that either represents the segment of missing bone or can be molded to fit the missing segment or juxtapose the ends of the bone. The cells are induced to form bone ex vivo using appropriate cell culture conditions or with inductive factors, such as bone morphogenetic protein-2 (BMP-2). Once the cells have begun to elaborate a new bone matrix, the construct can be implanted into the patient to effect osteogenesis and promote healing. This sequence of events is typically referred to as a tissue engineering approach to enhancing osteogenesis.

[0093] Inhibition of 5-lipoxygenase (5-LO) can be used to promote ex vivo bone formation for tissue engineering application. This is accomplished by promoting osteogenesis ex vivo with small molecule inhibitors of 5-LO or FLAP alone or in combination with well known inductive agents, such as BMP-2.
[0094] A second approach uses RNAi technology to inhibit 5-LO activity and promote ex vivo osteogenesis. This is accomplished by transfecting the cultured precursor skeletal cells with pools of siRNA sequences using commercially available transfection reagents, such as TransIT-TKO or jetSI. Approximately 1 million cells are transfected with a cocktail of 3 siRNAs specific for 5-LO or FLAP using 50-200 pmoles of each siRNA. Alternatively, a pool of siRNAs that target 5-LO and FLAP is used. As a control, cells are transfected similarly with commercially available siRNAs developed to knock-down enhanced green fluorescent protein (EGFP). Knock-down of 5-LO or FLAP is confirmed by western blot analysis and the results quantified to insure a greater than 80% reduction in 5-LO and/or FLAP expression.

[0095] The treated precursor skeletal cells are cultured and osteogenesis is assessed as extracellular matrix production of cartilage or bone matrix using measures such as alcian blue or alizarin red binding as appropriate or measures of specific matrix protein. Knock-down of 5-LO or FLAP promotes osteogenesis based upon enhanced calcified matrix deposition measured by alizarin red binding. This indicates that an RNAi or anti-sense approach to inhibiting 5-LO activity is useful for promoting osteogenesis ex vivo for purposes of tissue engineering.

[0096] Pools of siRNA pairs for 5-LO can be chosen, e.g., from POOL-A (5'-AAC TGG GCG AGA TCC AGC TGG-3' (SEQ ID NO: 9), 5'-AAG CTC CCG GTG ACC ACG GAG-3' (SEQ ID NO: 10), 5'-AAG GAA GCC ATG GCC CGA TTC-3') (SEQ ID NO: 11), POOL-B (5'-AAT CGA GAA GCG CAA GTA CTG-3' (SEQ ID NO: 12), 5'-AAG GAG TGG ACT TTG TTC TGA-3' (SEQ ID NO: 13), 5'-AAC TTA GGC CAG TAC GAC TGG-3') (SEQ ID NO: 14), or POOL-C (5'-AAG TTG GCC CAA GAT GAC CAA-3' (SEQ ID NO: 15), 5'-AAC ACA TCT GTG GTC TGA GGT-3' (SEQ ID NO: 16), 5'-AAC CAT GCG AGC CCC GCC ACC-3') (SEQ ID NO: 17). Pools of siRNA pairs for FLAP can be chosen, e.g., from POOL-D (5'-AAC CAA ACA TGG ATC AAG AAA-3' (SEQ ID NO: 18), 5'-AAG TTC CTG CTG CCG TTG CTG-3' (SEQ ID NO: 19), 5'-AAT TCA GCT CTT GAG AGC ATT-3') (SEQ ID NO: 20), POOL-E (5'-AAT GGA TTC TTT GCC CAT AAA-3' (SEQ ID NO: 21), 5'-AAG TAC TTT GTC GGT TAC TTA-3') (SEQ ID NO: 22), 5'-AAT CTA TTG GCC ATC TGG GCT-3') (SEQ ID NO: 23), or POOL-F (5'-AAC CAG AAC TGT GTA GAT GCG-3' (SEQ ID NO: 24), 5'-AAG TGA CTT TGA AAA CTA CAT-3' (SEQ ID NO: 25), 5'-AAT GAT GTC ATG TCA GCT CCG-3') (SEQ ID NO: 26). For brevity, only the sense strand of each siRNA pair is shown. It is well known in the art that siRNA pairs are double stranded small RNAs that have a 5'-AA overhang on the sense strand.
and a 5'-UU overhang on the antisense strand. It also is well known in the art that backbone chemistry modifications can be advantageous for stabilizing or improving the uptake of the siRNA molecules. Pirollo KF et al., (2003), Rait A, Sleer LS, Chang EH, “Antisense therapeutics: from theory to clinical practice,” Pharmacol Ther. 99(1):55-77. Manufacture of oligonucleotides with advantageous backbone chemistry modifications is within the level of ordinary skill, and use of such modified–backbone compounds (as well as non-modified–backbone compounds) is within the scope of the present invention.

[0097] One skilled in the art will recognize that in addition to direct transfection of the siRNAs into cells, expression vectors can be developed that express these or similar sequences and the expression vectors delivered to the cells by transfection, viral mediated delivery, or methods for delivering DNA molecules into cells. The expression vectors express the siRNAs leading to sustained inhibition of 5-LO, FLAP, or both and thereby promoting osteogenesis.

[0098] One skilled in the art also will recognize that additional strategies to inhibit expression of 5-LO or FLAP can be used to promote the same osteogenic effects in the precursor skeletal cells. Such technologies include use of anti-sense.

[0099] Exemplary 5-Lipoxygenase anti-sense sequences include, e.g., 5'-GCA GGT GCT TCT CGC TGC AGC C-3' (SEQ ID NO: 27), 5'-GCC AGT ACT TGC GCT TCT CG-3' (SEQ ID NO: 28), 5'-CCA TCG ATA TTG TTT TTG CC-3' (SEQ ID NO: 29), 5'-GGA GCT TCT CGG GCA GCT CTG TGC-3' (SEQ ID NO: 30), 5'-CCA GGT TCT TAT ACA GCA AGC-3' (SEQ ID NO: 31), 5'-CCA GCA GCT TGA AAA TGG GGT GC-3' (SEQ ID NO: 32), 5'-GCC CCG GGC CCTT GAT GGC C-3' (SEQ ID NO: 33), 5'-CCA CGC CCT TGG CAG TCG C-3' (SEQ ID NO: 34), and 5'-GCG GAA TCG GGC CAT GGC TTC C-3' (SEQ ID NO: 35).

[0100] Exemplary FLAP anti-sense sequences include, e.g., 5'-GTT CCG GTC CTC TGG AAG CTC C-3' (SEQ ID NO: 36), 5'-CGC AGA CCA GAG CAC AGC G-3' (SEQ ID NO: 37), 5'-GCA AAC GCA GCA GGA AC-3' (SEQ ID NO: 38), 5'-CGT TTC CCA AAT ATG TAG CC-3' (SEQ ID NO: 39), 5'-GGT TTC AAA GTC ACT TCC G-3' (SEQ ID NO: 40), 5'-GGT TAA CTC AAG CTG TGA AGC-3' (SEQ ID NO: 41), 5'-GGA GCT GAC ATG ACA TC-3' (SEQ ID NO: 42), and 5'-GGC CAC GGT CAT GTT CAA GG-3' (SEQ ID NO: 43).

[0101] Thus, novel methods for promoting osteogenesis to accelerate or enhance bone fracture healing, treat bone defects, and enhance bone formation are disclosed. Although preferred embodiments of the subject invention have been described in some detail, it is
understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.
WE CLAIM:

1. A method for promoting osteogenesis to treat a mammalian subject in need thereof, comprising: administering to said subject a pharmaceutically effective amount of compound that reduces a 5-lipoxygenase activity, wherein said 5-lipoxygenase activity reduction promotes osteogenesis to treat a condition selected from the group consisting of a bone fracture, a bone defect, and a condition treated by inducing bone formation.

2. The method of claim 1, wherein said condition is a bone fracture.

3. The method of claim 2, wherein said bone fracture is a non-osteoporotic fracture, an osteoporotic fracture, a fracture associated with a congenital disease, a fracture associated with an acquired disease, or an osteotomic fracture.

4. The method of claim 3, wherein said bone fracture is a non-osteoporotic fracture.

5. The method of claim 3, wherein said bone fracture is an osteoporotic fracture.

6. The method of claim 3, wherein said bone fracture is an osteotomic fracture.

7. The method of claim 1, wherein said condition is a condition treated by inducing bone formation.

8. The method of claim 7, wherein said condition is a condition treated by a spine fusion, or a joint arthrodesis.

9. The method of claim 1, wherein said condition is a bone defect.

10. The method of claim 1, wherein said administration is in vivo.

11. The method of claim 1, wherein said administration is ex vivo.

12. The method of claim 1, wherein said compound reduces a 5-lipoxygenase activity by inhibiting a five lipoxygenase activating protein (FLAP).

13. The method of claim 1, wherein said compound comprises a small molecule.

14. The method of claim 13, wherein said small molecule is selected from the group consisting of 3-[1-(4-chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid (MK886); 3-(1-(4-chlorobenzyl)-3-(1-butyl-thio)-5-(quinolin-2-ylmethoxy)-indol-2-yl)-2,2-dimethyl propanoic acid (MK-591); nordihydroguaiaretic acid (NDGA); 2-(12-hydroxydoca-5,10-diynyl)-3,5,6-trimethyl-1,4-benzoquinone (AA861); (N-(1-benzo(b)thien-2-ylethyl)-N-hydroxyurea) (zileuton); masoprocol; tenidap; flobufen; lonapalene; tagorizine; AA-861; Abbott A-121798; Abbott A-76745; Abbott A-78773; [(R)(+)]N-[[5-(4-fluorophenoxo)furan-2-yl]-1-methyl-2-propynyl]-N-hydroxyurea (Abbott A-79175); Abbott ABT 761; Dainippon AL-3264; Bayer Bay-x-1005; Biofor BF-389; bunaprost; Cytomed CMI-392; Takeda CV-6504; enazadrem phosphate; Leo Denmark ETH-615; flezelastine hydrochloride; Merck Frosst L-663536; Merckle ML-3000; 3M
Pharmaceuticals R-840; rilpivirox; Schering Plough SCH-40120; tepoxalin; linazolast (TMK-688); Zeneca ZD-2138; Bristol-Myers Squibb BU-4601A; carbazomycin C; lagunamycin; Wellcome BW-70C; Ciba-Geigy CGS-26529; Warner-Lambert CI 1004; Warner-Lambert PD-136005; Warner-Lambert PD-145246; Elsali E-3040; Fujirebio F-1322; Fujisawa FR-110302; Merck Frosst L-699333; Merck Frosst L-739010; Lilly LY-269415; Lilly LY-178002; Hoechst Roussel P-8892; SmithKline Beecham SB-202235; American Home Products WAY-121520; American Home Products WAY-125007; Zeneca ZD-7717; Zeneca ZM-216800; Zeneca ZM-230487; 1,2-dihydro-n-(2-thiazolyl)-1-oxopyrrolo(3,2,1-kl)phenothiazine-1-carboxamide; Abbott A-65260; Abbott A-69412; Abbott-63162; American Home Products AHR-5333; Bayer Bay-q-1531; Boehringer Ingelheim BI-L-357; Boehringer Ingelheim BI-L-93BS; Boehringer Ingelheim BIL 226XX; Bristol-Myers Squibb BMY-30094; carbazomycin B; Wellcome BW-B218C; Chauvin CBS-1114; Ciba-Geigy CGS-21595; Ciba-Geigy CGS-22745; Ciba-Geigy CGS-23885; Ciba-Geigy CGS 24891; Ciba-Geigy CGS-8515; Chiesi CHF-1909; Warner-Lambert CI-986; Warner-Lambert CI 987; cirsiliol; docebenone; Eisai E-5110; Eisai E-6080; enofelast; epocarbazolin-A; eprovafen; evandamine; Fisons FPL 62064; Zeneca ICI-211965; Zeneca ICI-216800; Kyowa Hakko KF-8940; Merck & Co L-651392; Merck & Co L-651896; Merck & Co L-652343; Merck & Co L-656224; Merck & Co L-670630; Merck & Co L-674636; Lilly LY-233569; Merck & Co MK-591; Merck & Co L-655240; nitrosoxacin-A; Ono ONO-5349; Ono ONO-LP-219; Ono ONO-LP-269; Warner-Lambert PD-127443; Purdue Frederick PF-5901; Rhone-Poulenc Rorer Rev-5367; Rhone-Poulenc Rorer RG-5901-A; Rhone-Poulenc Rorer RG-6866; Roussel-Uclaf RU-46057; Searle SC-41661A; Searle SC-45662; Sandoz SDZ-210-610; SmithKline Beecham SK&F-104351; SmithKline Beecham SK&F-104493; SmithKline Beecham SK&F-105809; Synthelabo SL-81-0433; Teijin TEI-8005; Terumo TMK-777; Terumo TMK-781; Terumo TMK-789; Terumo TMK-919; Terumo TMK-992; Teikoku Hormone TZI-41127; American Home Products WAY-120739; American Home Products WY-47288; American Home Products WY-48252; American Home Products WY-50295; Yoshitomi Y-19432; 4-[3-[4-(2-methyl-1H-imidazol-1-yl)phenyl(thio)]phenyl-3,4,5,6-tetrahydro-2H-pyran-4-carboxamide; esculetin; phenidone; BI-L-239; 5,8,11-eicosatriynoic acid (ETI); 5,8,11,14-eicosatetraynoic acid (ETYA); cinnamyl-3,4-dihydroxy-alpha-cyanocinnamate; curcumin; esculetin; gossypol; caffeic acid; baicailein; 7,7-dimethyleicosadrenoic acid (DEDA); Ly311727; bromoenol lactone; methyl arachidonoyl fluorophosphonate; methyl y-linolenyl fluorophosphonate; oleoyxyethyl phosphorylcholine; AACOF3; n-(p-amylcinnamoyl) anthranilic acid; mepacrine; quinacrine; atabrine;
parabromophenacylbromide; aristolochic acid; cortisone Glaxo SmithKline 480848; Glaxo SmithKline 659032; Glaxo SmithKline 677116; BMS-181162; MJ33; and Millennium Pharmaceuticals MLN977.

15. The method of claim 14, wherein said small molecule is selected from the group consisting of masprocol; tenidap; (N-(1-benzo(b)thien-2-ylethyl)-N-hydroxyurea) (zileuton); flubufen; lonapalene; tagorizine; AA-861; Abbott A-121798; Abbott A-76745; Abbott A-78773; [(R)(+)]N'-[[5-(4-fluorophenoxy)furan-2-yl]-1-methyl-2-propylnyl]-N-hydroxyurea (Abbott A-79175); Abbott ABT 761; Dainippon AL-3264; Bayer Bay-x-1005; Biofor BF-389; bupaprolast; Cytomed CMI-392; Takeda CV-6504; Ciba-Geigy CGS-26529; enazadrem phosphate; Leo Denmark ETH-615; flezelastine hydrochloride; Merck Frost L-663536; Merck Frost L-699333; Merckle ML-3000, 3M Pharmaceuticals R-840; rilopirox; Schering Plough SCH-40120; tepoxalin; linazolast (TMK-688); Zeneca ZD-7717; Zeneca ZM-216800; Zeneca ZM-230487; Zeneca ZD-2138; and NDGA (nondihydroguaiaretic acid).

16. The method of claim 15, wherein said small molecule is selected from the group consisting of tenidap; zileuton; flubufen; lonapalene; tagorizine; AA-861; Abbott A-121798; Abbott A-76745; Abbott A-78773; [(R)(+)]N'-[[5-(4-fluorophenoxy)furan-2-yl]-1-methyl-2-propylnyl]-N-hydroxyurea (Abbott A-79175); Abbott ABT 761; Ciba-Geigy CGS-26529; Biofor BF-389; Cytomed. CMI-392; Leo Denmark ETH-615; Merck Frost L 699333; Merckle ML-3000; 3M Pharmaceuticals R-840; linazolast (TMK-688); Zeneca ZD-7717; Zeneca ZM-216800; Zeneca ZM-230487; Zeneca ZD-2138, and NDGA (nondihydroguaiaretic acid).

17. The method of claim 11, wherein said compound comprises a nucleic acid comprising a sequence selected from the group consisting of 5'-AAC TGG GCG AGA TCC AGC TGG-3', 5'-AAG CTC CCG GTG ACC ACG GAG-3', 5'-AAG GAA GCC ATG GCC CGA TTC-3', 5'-AAT CGA GAA GCG CAA GTA CTG-3', 5'-AAG GAG TGG ACT TTG TTC TGA-3', 5'-AAC GTG GGC CAG TAC GAC TGG-3', 5'-AAG TTG GCC CGA GAT GAC CAA-3', 5'-AAC ACA TCT GTT GTC TGA GTG-3', 5'-AAC CAT GCG AGC CCC GCC ACC-3', 5'-AAG CAA ACA TGG ATC AAG AAA-3', 5'-AAG TCC CTG CGT TGG CTG-3', 5'-AAT TCA GCT CTT GAG AGC ATT-3', 5'-AAT GGA TCC TTT GCC CAT AAA-3', 5'-AAG TAC TTT GTC GGT TAC CTA-3', 5'-AAT CTA TTG GCC ATC TGG GCT-3', 5'-AAC CAG AAC TGT GTA GAT GCG-3' 5'-AAG TGA CTT TGA AAA CTA CAT-3', and 5'-AAT GAT GTC ATG TCA GCT CCG-3'.

18. The method of claim 11, wherein said compound comprises a nucleic acid comprising a sequence selected from the group consisting of 5'-GCA GGT GCT TCT CGC TGC AGC
C-3', 5'-GCC AGT ACT TGC GCT TCT CG-3', 5'-CCA TCG ATA TTG TTG CC-3', 5'-GGA GCT TCT CGG GCA GCT CTG TGC-3', 5'-CCA GGT TCT TAT ACA GCA AGC-3', 5'-CCA GCA GCT TGA AAA TGG GGT GC-3', 5'-GCC CCG GGC CTT GAT GGC C-3', 5'-CCA CGC CCT TGG CAG TCG G-3', 5'-GCG GAA TCG GGC CAT GGC TTC C-3', 5'-GTT CCG GTC CTC TGG AAG CTC C-3', 5'-CGC AGA CCA GAG CAC AGC G-3', 5'-GCA AAC GCA GCA GGA AC-3', 5'-CGT TTC CCA AAT ATG TAG TAG CC-3', 5'-GTT TTC AAA GTC ACT TCC G-3', 5'-GGT TAA CTC AAG CTG TGA AGC-3', 5'-GGA GCT GAC ATG ACA TC-3', and 5'-GGC CAC GGT CAT GTT CAA GG-3'.

19. The method of claim 1, further comprising administering to said subject a pharmaceutically effective amount of a compound that reduces a COX-1 activity.

20. The method of claim 19, wherein said compound is selected from the group consisting of SC-560, FR122047, Valeroyl salicylate, Aspirin, Dexketoprofene, Keterolac, Flurbiprofen, and Suprofen.

21. The method of claim 1, further comprising administering to said subject a pharmaceutically effective amount of a compound that increases a COX-2 activity.

22. The method of claim 21, wherein said compound is selected from the group consisting of Prostaglandin E2, butaprostone, sulprostone, CP-536,745-01, CP-043,305-02, CP-044,519-02, CP432, ONO-4819, CP-533,536, prostaglandin F2alpha, bimatoprost, cloprostenol, latanoprost, tafluprost, bone morphogenetic protein-2 (BMP2), platelet derived growth factor (PDGF), interleukin-1alpha, interleukin-1beta, tumor necrosis factor-alpha (TNF-alpha), fibroblast growth factor (FGF), transforming growth factor-beta (TGF-beta), epidermal growth factor (EGF), parathyroid hormone (PTH), parathyroid hormone related peptide (PTHrP), and teraparatide.

23. The method of claim 1, comprising administering to said subject an ultrasound therapy or exposing said subject to a pulsed electromagnetic field in an amount sufficient to increase a COX-2 activity in said subject.
FIGURE 1

Arachidonic Acid Metabolism and Signaling

Phospholipase A2

Arachidonic Acid

Cyclooxygenase

5-Lipoxygenase

PGH2

PGD2

PGE2

PGF2a

PGI2

TXA2

5-HETE

LTA4

LTC4

LTD4

LTE4

LXA4

LXB4

12-LOX

15-LOX

BLT1

BLT2

CysLT1

CysLT2

ALX/R

(+cAMP

(-cAMP

(+Ca^{2+}

(+Ca^{2+}

EP1, EP2, EP4

EP3

EP1, FP, TP

(+cAMP

(-cAMP
FIGURE 2

A. Normal Healing: Balanced COX-2 and 5-LO

B. Impaired Healing: COX-2 Insufficiency

C. Enhanced Healing: 5-LO Insufficiency
FIGURE 7

Rat A (control),
3 weeks post-fracture

Rat B (Control),
3 weeks post-fracture

Rat C (5-LO inhibitor treated),
3 weeks post-fracture

Rat D (5-LO inhibitor treated),
3 weeks post-fracture
FIGURE 8

Vehicle    NDGA    AA-861

8A        8B        8C

8D
Inhibition of 5-LO Increases Fracture Callus Peak Torque

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