5-Lipoxygenase: The Therapeutically Unexplored Target for Bone Health

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Abstract

Bone fracture is a common orthopedic condition that represents a significant health concern. Bone fracture repair process is a complex process that involves the harmonic and synchronized activity of bone cells. 5-lipoxygenase (5-LOX) is an enzyme responsible for the conversion of arachidonic acid to form leukotrienes. While leukotrienes have an empirical inflammatory role, various evidence suggests that these 5-LOX mediators, by switching the inflammatory environment and altering the activity of bone cells, have a detrimental effect on the bone fracture healing process. Additionally, various evidence suggests that 5-LOX inhibition shows improvement in the overall bone healing process and improves overall bone health. Despite this evidence, the clinical use of 5-LOX inhibitors in bone fracture healing is largely unexplored. The current review aimed to summarize the available evidences and pave the way for future large scale pre-clinical and clinical studies to evaluate the effectiveness of selective 5-LOX inhibitors in bone fracture healing. A comprehensive literature search was conducted in PubMed, Google Scholar, and ScienceDirect to identify relevant articles related to the effect of 5-LOX in bone health. The summary of available scientific evidence highlights that the selective 5-LOX inhibition can modulate the functioning of osteoclasts and osteoblasts, favoring faster bone fracture healing. The observations of this study support the selective 5-LOX inhibition as a potential treatment option that can improve bone fracture healing rate and therefore future clinical studies can be designed to confirm the observations of the current review study.

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Keywords: Lipoxygenase; Bone Fracture; Leukotrienes

Introduction

Bone, a type of connective tissue and the major component of the skeletal system, is responsible for providing support, protection, facilitating movements and locomotion, and serving as a mineral reservoir for essential elements, mainly calcium and phosphorus (1,2). Bone is a dynamic tissue that is broken down and formed continuously, either as part of the bone repair process or to maintain the normal homeostasis of the body (3). While the normal physiological process of bone breakdown and formation is a relatively simple and straightforward process, the bone healing process after bone fracture is a completely different and complex procedure involving various mechanisms working simultaneously to provide the complete structural and mechanical recovery of bone back to its normal state (4,5).

Bone fracture, referred to as a partial or complete break in the continuity of the bone, is one of the most common orthopedic conditions (6,7). Bone fractures can be caused due to numerous reasons, the major risk factors include accidental slips, trauma (accidents), sports injuries, and certain disease conditions (like osteoporosis and sarcopenia) (Figure 1) (8–11). As bone fracture is one of the most common orthopedic conditions, the exact prevalence is difficult to estimate, with around 170 million individuals having bone fracture globally per year which poses a severe economic burden (exceeding billions of dollars), productivity losses, and physical disability of individuals (6,7,12). Bone fracture is commonly associated with severe pain, physical impairment, social isolation, and low self-esteem (13).

While the majority of fractures are treated by restricting injured site mobility with the casting method, certain complex fractures require surgical interventions like the surgical introduction of locking plates, arthroplasty (if the entire joint is damaged along with the bone fracture), and the use of intramedullary nails for fixing bones with each other (14). Additionally, various non-pharmacological treatment options (including physiotherapy and home exercise) are also initiated to improve the overall physical and mental health of individuals (15–17). Various pharmacological interventions are also provided with the main objective of improving the bone healing rate, which may lead to faster

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bone healing. Pharmacological therapies widely used in bone fracture include calcium and vitamin D supplements (to improve bone mineralization rate) (18), anabolic agents like teriparatide (to stimulate bone formation) (19), anti-resorptive agents like bisphosphonates (to prevent further bone loss, particularly useful in osteoporosis) (20), monoclonal antibody therapies like denosumab (to hamper bone breakdown) (21), and hormonal therapies including calcitonin and estrogen (22–24). Various herbal supplements, including the supplementation of Cissus quadrangularis extract, are also used in the treatment of bone fractures (25). While many treatment options are currently being employed for bone fracture healing, several bone fractures end in non-union conditions (prolonged incomplete healing of fractured bone) (6,26). Hence, understanding the normal bone healing process and the role of individual phases of bone healing on overall bone health is crucial for identifying new therapeutic targets that can improve bone healing rate.

Fracture healing process

Bone fracture healing processes are of two types: primary (direct) healing and secondary (indirect) healing process (26). While the primary bone healing process is a relatively uncommon process that occurs under the pressure of rigid fixation and majorly involves the bridging of Haversian systems (or osteons), the secondary bone healing process is a relatively complex process that involves various systemic and cellular changes that work in harmony that leads to complete bone recovery to original shape, size, strength (26–28). The bone fracture healing process is divided into four stages: the inflammatory phase, soft callus formation, hard callus formation, and the remodeling phase (11).

Inflammatory phase

The inflammatory phase is the first defense and healing mechanism initiated immediately after a bone fracture (26). Initially, the rupture of blood vessels causes the formation of blood clots around the injured site (known as hematoma). The hematoma forms a complex microenvironment at the fracture site, functions to physically lock the injured sites from movement and serves as the platform for further bone fracture healing process (29).

Leukocytes from the bone marrow are the initial immune cells that get activated and spread in the injured site and surrounding area and release various cytokines that initiate the inflammatory phase and cause the immigration of more immune cells (particularly neutrophils and macrophages) to the injured site to initiate further healing process (30–32). Immunohistochemical studies have identified the presence of arachidonic acid inflammatory pathway enzymes (namely cyclooxygenase (COX) and 5-lipoxygenase (5-LOX)) to be majorly present at various immune cells (majorly leukocytes) around injured sites, indicating a major role of these metabolites in bone fracture healing (32). While previous studies have demonstrated an important role of COX and its mediators in bone fracture healing (32,33), the addition of leukotriene B4 (LTB4) also demonstrated that it altered the differentiation and proliferation rate of osteoblasts (34). These data indicate the complex role of COX and 5-LOX metabolites during the inflammatory phase.

Soft callus formation (Cartilaginous callus)

Various growth factors, such as transforming growth factor-beta (TGF-β), fibroblast growth factor (FGF), and insulin-like growth factors (IGFs), get activated and increase the proliferation and differentiation of specialized cells like fibroblasts and chondrocytes that migrate to the fracture site and create a soft callus (majorly composed of collagen and cartilage) to provide initial stability to the fractured bone (35). The soft callus helps bridge the fracture gap and serves as a precursor to the subsequent bone-forming phase (35).

Hard callus formation (Endochondral ossification)

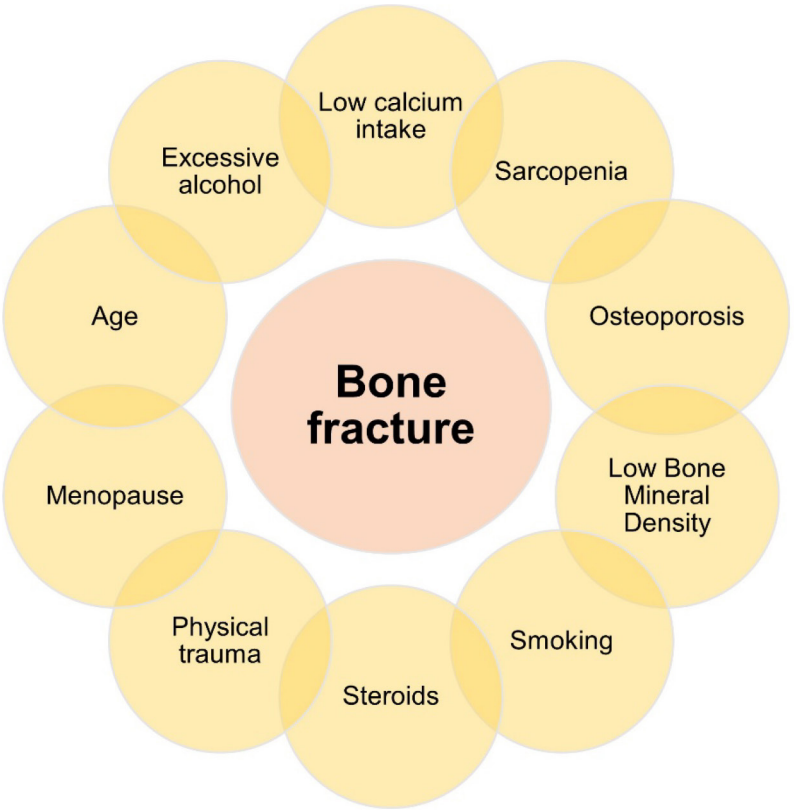
The formation of a soft callus provides the framework for the migration of osteoprogenitor cells (OPCs) (derived from mesenchymal stem cells (MSCs)) from the bone marrow to the injured site. These OPCs further differentiate to form osteoblasts, which function primarily to deposit calcium and phosphate on the soft callus (a process called callus mineralization) ultimately causing the hardening and calcification of the matrix to form the hard callus (35). During this stage, various growth factors, (including bone morphogenetic proteins (BMPs)) play a pivotal role in regulating osteoblast formation, bone matrix formation, and vascularization (35). While the effect of 5-LOX on the soft and hard callus formation stage has not been fully explored, previous studies have noted that the expression of 5-LOX remains high even after the resolution of inflammation at the injured site, while other studies have identified that cysteinyl leukotrienes have a negative influence on the function of MSC (26,36). These data indicate that the negative influence of 5-LOX-mediated inflammation may be extended to other later phases of bone healing as well.

Remodeling phase

The final stage of fracture healing involves the remodeling of the hard callus into mature bone (35). While the complete, well-organized bone remodeling process is

not within the scope of the current review, it involves the initial bone resorption by osteoclasts, followed by new bone formation by osteoblasts, which plays a crucial role in maintaining bone integrity (35).

Figure 1. Risk factors of bone fracture



Method of literature review

The current review article was conducted to evaluate the effect of 5-LOX and their metabolites in bone fracture healing rate and overall bone health. For this purpose, we conducted an in-depth literature search in three online databases (PubMed, Google Scholar, and ScienceDirect) from inception till October 2023. Articles of clinical and preclinical studies that evaluated the role of 5-LOX in bone health were identified and evaluated. The databases search was conducted by using a combination of Medical Subject Heading (MeSH) terms along with free-text words that were related to the effect of 5-LOX on bone health.

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5-LOX and bone fracture healing

5-LOX, belonging to the leukotriene family, is made of 673 amino acids with a molecular weight of 78kDA (37–39). 5-LOX plays an important role in various inflammatory phases, and it is also involved in various chronic inflammatory disease conditions, including osteoarthritis, inflammatory bowel disease, asthma, allergy, chronic obstructive pulmonary disease, cardiovascular conditions (like atherosclerosis), oncologic conditions, and COVID-19 as well (40–44). Additionally, several studies have explored the potential role of 5-LOX in normal bone remodeling and various stages of bone fracture healing.

5-LOX metabolites effect on osteoclasts

Four scientific studies have assessed the impact of 5-LOX metabolites on osteoclast functioning, as summarized in Table 1. The initial study, which primarily focused on identifying the key elements responsible for the activity of osteoclasts, was evaluated using the C433 osteosarcoma cell-line (45). The study utilized the C433 osteosarcoma cells, isolated the key mediators produced by these cells, and studied the stimulatory or inhibitory effect of these mediators on rodent osteoclasts and subsequent osteolytic activity. Among the various mediators, the cell line majorly produced 5-hydroxyeicosatetraenoic acid (5-HETE) and leukotrienes that triggered the osteoclasts and increased their activity. The addition of these mediators to avian osteoclast cell culture increased the proliferation of osteoclasts, while the addition of these mediators to rodent bone lining showed increased formation of bone lesions

and resorption pits due to increased osteoclasts-mediated bone resorption. Additionally, the addition of selective COX inhibitors showed no change in the C433-mediated osteoclastic activity, while the addition of selective 5-LOX inhibitor dose-dependently prevented the bone resorptive activity. While the study failed to identify the exact mechanism of action of 5-LOX metabolites on osteoclasts, the results indicated that 5-LOX metabolites are primarily responsible for the altered activity of osteoclasts. Furthermore, the level of these metabolites was observed to have a dose-dependent negative impact on osteoclast-mediated bone healing (45).

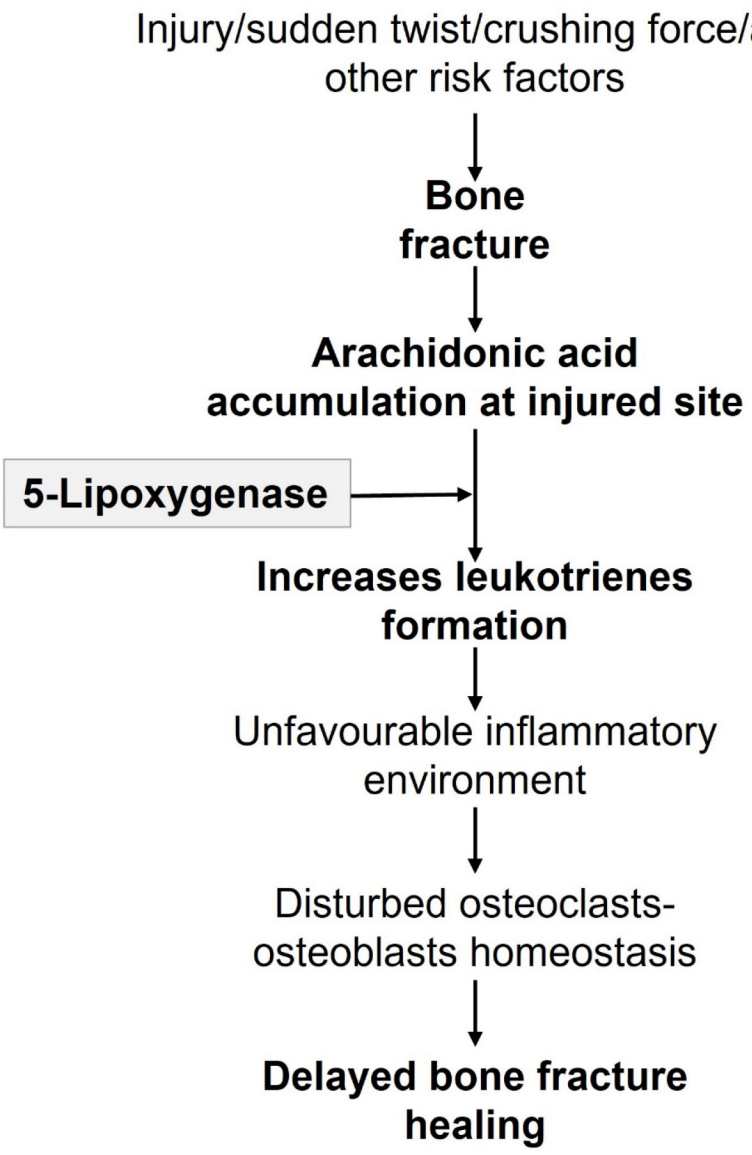
In a further study using mouse bone marrow-derived macrophages (BMMs), the molecular role of 5-LOX in osteoclastogenesis was evaluated (44). Specific 5-LOX inhibitor (MK886) and cysteinyl leukotriene receptor (CysLTR) inhibitor (REV5901) were utilized and their inhibitory effect on osteoclastogenesis was evaluated using BMM-cells treated with receptor activator of nuclear factor-κ ligand (RANKL). Treatment of BMM cells with RANKL resulted in osteoclastogenesis, while both 5-LOX and CysLTR inhibitors modulated osteoclastogenesis in a dose-dependent manner. Similarly, the treatment of 5-LOX and CysLTR inhibitors prevented the formation of resorption pits, indicating altered activity of pre-formed osteoclasts. To evaluate the genetic basis of the effect of 5-LOX and CysLTR inhibitors on osteoclastogenesis, a gene expression study was conducted, which showed that treatment of BMMs with RANKL increased the expression of various genes (including genes that express for cathepsin K and integrin αv/ß3) related to osteoclast functioning, while treatment with 5-LOX and CysLTR inhibitors altered the gene expression in a dose-dependent manner in a favorable manner that prevented the formation of resorptive pits. The addition of LTC4 to the 5-LOX and CysLTR-treated cells reversed the modulatory effect and increased osteoclast-related gene expression. These data suggest that 5-LOX metabolites have an important role in osteoclastogenesis and osteoclast activity by altering various gene expressions (44).

The molecular effect of 5-LOX and CysLTR inhibitors on osteoclastogenesis was evaluated by identifying the molecular signaling pattern (44). In normal condition, the binding of RANKL to the RANK receptor induces signaling pathways (mainly Extracellular signal-regulated kinase (ERK), Mitogen-activated protein kinase (MAPK), Jun N-terminal kinase (JNK), and p38 pathways) that trigger the activation of nuclear factor-κB (NF-κB) and c-Fos which in turn increases the expression of NFATc1, a key regulator of osteoclastogenesis and osteoclast activity (46). Treatment of BMMs with RANKL significantly increased the ERK and p38 downstream signaling and increased the activity of NF-κB, while treatment with 5-LOX and CysLTR inhibitors prevented the overactivation of these pathways. Similarly, the expression of NFATc1 was significantly increased after RANKL treatment, and it was controlled after treatment with 5-LOX and CysLTR inhibitors. The addition of exogenous NFATc1 to 5-LOX and CysLTR inhibitor-treated cells partially reversed the inhibitory effect on osteoclastogenesis, which confirms that 5-LOX and CysLTR inhibitors might improve bone fracture healing rate partly via altering NFATc1 expression. These results were further confirmed by performing 5-LOX and CysLTR knockout in BMM cells using short-interfering (or silencing) RNA. The knockout of 5-LOX and CysLTR showed similar effects on osteoclastogenesis and osteoclast activity, like the exogenous addition of 5-LOX and CysLTR inhibitors. All this evidence points towards the potential role of 5-LOX and its metabolites in osteoclastogenesis and bone resorption activity, and the study underscores the potential of 5-LOX inhibition as a treatment option that can be explored in disease conditions associated with increased bone resorption, like osteoporosis (44).

While the in-vitro studies provide prominent evidence regarding the role of 5-LOX in osteoclastogenesis, limited in-vivo studies have been conducted to evaluate and confirm these findings. In a study involving C57BL/6 mice, apical periodontitis (AP) was induced to evaluate the effect of 5-LOX inhibitors on osteoclast activity and subsequent bone resorption rate (47). As per the hypothesis, the AP induction caused increased expression of 5-LOX and associated mediators in the affected tooth environment. While the use of MK886 resulted in 5-LOX inhibition and subsequently prevented the bone resorption phase, this effect was only evident during the early phase of infection. At the late stage of infection, bone resorption was found to be evident even in the presence of MK886. This effect was observed due to the body's immune system activation, causing an increase in the level of inflammatory cytokines and chemokines from the systemic circulation to the infected tooth environment. The influx of these inflammatory cytokines resulted in the secondary activation of osteoclastogenesis, and that was further responsible for observed bone resorption during the late phase of infection. The results of this study were partly in line with the results of previous in-vitro evidence that 5-LOX inhibition can prevent bone resorptive rate via modulating osteoclast activity (44,45). Additionally, the study highlights the role of cytokines and chemokines present in the systemic circulation in the secondary osteoclastogenesis

process and subsequent bone resorption. Hence, it can be visualized that rather than local inhibition of 5-LOX, systemic inhibition of 5-LOX might be a potential way to alter osteoclastogenesis and subsequently prevent bone resorption in bone resorptive disease conditions (47). This hypothesis was confirmed in a lipopolysaccharide (LPS)-induced periodontal disease model (48). Using LPS injections, periodontitis was induced in mice, and the effect of an orally active systemic 5-LOX inhibitor (CJ-13610) on the overall inflammatory phase, osteoclastogenesis, osteoclast activity, and subsequent bone resorption level was evaluated. In line with the hypothesis generated from the previous study (47), the administration of CJ-13610 resulted in a significant reduction in the level of LTB4, which in turn reduced the level of inflammatory mediators in gingival tissues. The increased activity of osteoclasts was significantly prevented which was accompanied by a significant reduction in the level of bone resorption (48).

Figure 2. The pathophysiological aspect of 5-LOX in delaying bone fracture healing process



5-LOX metabolites effect on osteoblast

As studies have confirmed the positive influence of 5-LOX on osteoclastogenesis, osteoclast activity, and bone resorption rate, the research regarding the effect of 5-LOX on osteoblasts is still in its infancy, with very limited studies conducted. Only two studies evaluated the effect of 5-LOX metabolites on osteoblasts, which are briefly described in Table 2.

The first study to evaluate the effect of 5-LOX metabolites on osteoblasts can be dated back to 1991 (34). The study utilized LTB4 and LTC4 and evaluated their effect on normal osteoblast cell lines obtained from rat calvaria and human osteosarcoma cell lines. As per the hypothesis, LTB4 exerted a dose-dependent inhibitory effect on osteoblast DNA synthesis, and this effect was potentiated with the addition of a COX inhibitor (indomethacin), suggesting that COX inhibition potentiates LTB4's effect on osteoblasts. In contrast, LTC4 did not significantly affect osteoblast proliferation. Moreover, the addition of 5-LOX inhibitor (AA-861) showed no effect on the activity of LTB4. Among the various possible reasons, the most interesting possibility for the absence of a 5-LOX inhibition effect on LTB4 action is that the osteoblasts are more susceptible to the metabolites of 5-LOX, than to 5-LOX itself, and hence the presence of a 5-LOX inhibitor had no effect on osteoblast proliferation as the major metabolite of 5-LOX was previously present in the cell-line environment. As these results were too preliminary to provide a general theoretical statement, the findings of this study focused on the relationship between leukotrienes, COX inhibition, and osteoblast functioning (34).

Another in-vitro study investigated the impact of leukotrienes and peptide-leukotrienes on bone metabolism (49). Using fetal rat calvaria cells, dexamethasone, and recombinant human bone morphogenetic protein-2 (rhBMP-2), the effects of 5-HETE, LTB4, and LTD4 on bone nodule formation, alkaline phosphatase (ALP) activity, and cell proliferation were evaluated. As per the theory, the presence of dexamethasone and rhBMP-2 accelerated the bone formation process, as observed by an increase in the size of bone nodules, while the addition of 5-HETE and LTB4 reduced this activity in a dose-dependent manner. Similar dose-dependent action was observed in the ALP activity of cells treated with dexamethasone and rhBMP-2. The 5-HETE treatment resulted in a decrease in dexamethasone-treated fetal calvaria cell growth, while the rhBMP-2 treated cell culture showed no difference in cell number when treated with 5-LOX metabolites. In mice calvaria cell culture, rhBMP-2 stimulated bone formation, and further treatment with 5-HETE and LTB4 reduced this activity. The activity of 5-LOX metabolites was associated with morphological changes in osteoblasts, which suggests that the 5-LOX metabolites act to alter the bone formation process by causing morphological alteration in osteoblasts (49).

Role of 5-LOX in bone formation

The putative effect of 5-LOX and its metabolites on overall bone healing is presented in Figure 2. Based on the preliminary evidence of the effect of 5-LOX on osteoclasts and osteoblasts functioning, various animal in-vivo studies were conducted to investigate the role of 5-LOX on overall bone health, either using experimentally induced fracture or bone defect models,[50–54] or in experimentally induced osteoporotic models (55,56).

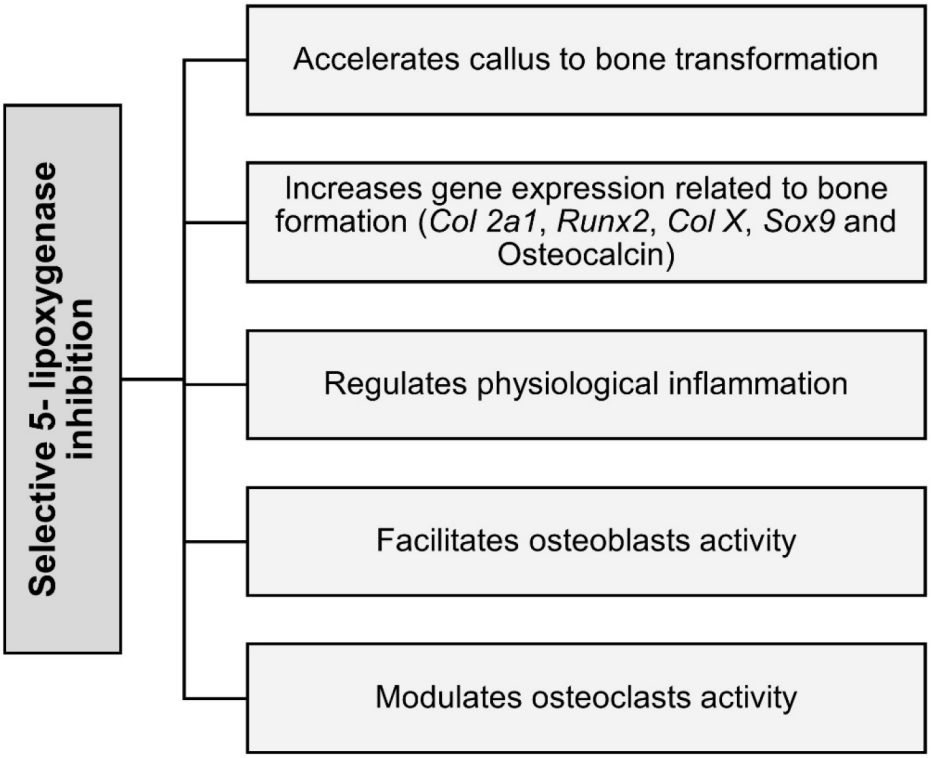
In a study evaluating the effect of 5-LOX in animal models with closed femoral fractures (50), the 5-LOX knockout mice were found to have accelerated bone fracture healing compared to wild-type mice of the same group. The callus in 5-LOX knockout mice transitioned from cartilage to bone faster, resulting in improved mechanical properties after one month of healing. Notably, callus leukotriene levels were significantly higher in COX-2 knockout mice, suggesting that in the absence of COX-2, arachidonic acid was directed toward the 5-LOX pathway. This data suggests that 5-LOX negatively affects fracture healing, and arachidonic acid's shift to the 5-LOX pathway might contribute to impaired healing in COX-2 knockout mice (50). In another study involving mice with closed femoral fractures, the effect of montelukast sodium (cysteinyl leukotriene type 1 receptor antagonist) and zileuton (5-LOX inhibitor) on the overall bone fracture healing rate was evaluated (51). In line with the previous results, the supplementation of montelukast sodium and zileuton improved the fracture healing rate. Both therapies showed a significant improvement in bone formation rate compared to the control group by significantly reducing the gene expression of cystLT1 and 5-LOX mRNA levels and increasing the expression of various bone-formation-related genes *(Col 2a1, Runx2, Sox9, Col X, and Osteocalcin)* (51).

In another study involving animals with bilateral femoral defects induced by speed drill (52), the application of a scaffold containing a 5-LOX inhibitor (A-79175) on the defected site showed a significant improvement in bone formation rate as compared to control scaffold animals. The bone healing improvement was observed in a dose-dependent manner with A-79175, indicating that 5-LOX inhibition can potentially accelerate bone fracture healing (52). Similar results were observed in an in-vivo animal study involving rats with femoral fractures (53). The administration of AA-861 was associated with significantly accelerated fracture healing, reducing fracture-bridging time, and increasing early callus cartilage and bone formation, along with improved mechanical properties. In the same study, the administration of a COX-2 inhibitor (celecoxib) showed inverse action and resulted in delayed fracture healing. While this study supports the theory of the detrimental effect of 5-LOX on bone fracture healing, the impairment of bone fracture

healing by COX inhibition was something additional that was particularly highlighted by this study (53). In a similar study involving wild and 5-LOX knockout mice (5-LOX^{KO} mice) with femur fractures, the 5-LOX^{KO} mice exhibited a faster bone formation rate as compared to wild mice (54). The 5-LOX^{KO} mice were found to have higher Runx2 gene expression as compared to wild mice. As the Runx2 gene is responsible for the formation of the Runx2 protein which is a key regulator of mesenchymal differentiation to immature osteoblasts, the results of the study highlight the potential inhibitory role of 5-LOX in mesenchymal cell differentiation and osteoblastogenesis (54,57,58).

The role of 5-LOX in overall bone health and bone-resorptive disease conditions was evaluated using female ovariectomized animal models (55,56). In an in-vivo study involving female ovariectomized (OVX) rats, the role of baicalein (5-LOX inhibitor) was evaluated (55). Administration of baicalein was associated with improved tibial density and cortical bone width compared to the control group (55). Similarly, the effect of zileuton and baicalein was evaluated in OVX rats. Both therapies were associated with an enhanced callus formation rate (56). All this evidence suggests that 5-LOX plays an important regulatory role in overall bone health. A summary of the studies regarding the effect of 5-LOX in bone formation is depicted in Table 3, while the potential mechanism of action of 5-LOX inhibitors in improving bone fracture healing rate and overall bone health is presented in Figure 3.

Figure 3. Putative mechanism of action of selective 5-LOX inhibition in improving bone fracture healing rate



Current therapeutic targets: Synergizing with 5-LOX inhibition

Bone fracture treatment options vary depending on the severity and type of fracture. In complex fractures, conservative approaches such as immobilization with casts, splints, or intramedullary nails may be used to heal the bone fracture (14). These surgical interventions work by stabilizing the fracture and aid in supporting the loads on the fractured bone, ultimately allowing the secondary healing process (59,60). Apart from surgical therapies, various pharmacological interventions are used to aid the bone fracture healing process, of which calcium and vitamin D supplements are the most commonly used interventions (18). Bone is mostly composed of hydroxyapatite (which is mostly composed of calcium and phosphorous), and during the bone fracture healing process, the calcium supplements are provided to ensure optimal calcium levels in the body for maintaining

an optimal callus mineralization rate (18). Vitamin D supplementation is done to ensure that sufficient vitamin D status is available in the body. Vitamin D has a crucial function in improving systemic calcium levels, which it does by increasing the intestinal calcium absorption rate (61). Anabolic agents like teriparatide are used to stimulate the bone formation rate by increasing osteoblast function and inhibiting the apoptosis of osteoblasts (19). Monoclonal antibody therapies like denosumab work by irreversibly binding to the RANKL and thereby preventing its association with RANK on macrophages, hence working as a decoy-therapy to prevent osteoclastogenesis and therefore prevent bone breakdown and promote bone formation (21). Additionally, hormonal therapies like calcitonin, which works by increasing calcium deposition in the bones and so improves the bone turnover rate, and estrogen supplementation, which is mostly used in postmenopausal women, prevent menopause-related osteoporosis, and improve the morphology of chondrocytes, thereby promoting faster fracture healing (22,23). Herbal therapies like *Cissus quadrangularis* are also used, which work by promoting matrix mineralization, improving osteoblast proliferation rate, and increasing the level of osteocalcin (62). While all these therapies have a clinically validated role in various aspects of bone fracture healing, none have a role in improving the inflammatory environment around fractures to improve bone fracture healing rates. The observations of the current review study suggest that inhibition of 5-LOX can potentially enhance bone fracture healing by regulating the fracture inflammatory environment, which thereby improves the function of osteoblasts, increases bone formation biomarkers, improves early callus formation rate, and enhances bone mineral density (44,51–55). Such observations underline the possibility that therapies (like selective 5-LOX inhibition) can act synergistically with conventional therapies, by inhibiting the detrimental role of 5-LOX and improving the overall bone healing rate. Such fascinating assumptions are required to be validated by future preclinical and clinical studies.

Table 1. Effect of 5-LOX metabolites on the osteoclast function

Author and Year	Study design	Animals/cell-line used	Methodology description	Study outcome
Gallwitz WE 1993 [45]	In-vitro	C433 osteosarcoma cell-line	To investigate the effect of mediators produced by osteosarcoma cells on different osteoclast cell cultures.	Mediators like 5-HETE and leukotrienes increased osteoclast-mediated bone resorption that was inhibited by the addition of a 5-LOX inhibitor.
Lee JM 2012 [44]	In-vitro	Mouse BMMs	To evaluate the molecular role of 5-LOX in osteoclastogenesis using specific 5-LOX and cysteinyl leukotriene inhibitors	5-LOX and CysLTR inhibitors reduced osteoclast activity and altered gene expression related to osteoclast function and molecular signaling pathways (ERK, p38, NF-κB, c-Fos, *NFATc1*).
Paula-Silva FWG 2016 [47]	In-vivo	C57BL/6 mice	To assess the impact of 5-LOX inhibitors on osteoclast activity and bone resorption rate using apical periodontitis model.	The 5-LOX inhibitor prevents early-phase bone resorption; systemic 5-LOX inhibition prevents osteoclastogenesis and bone resorption.
Lopes DEM 2018 [48]	In-vivo	Balb/c mice	To evaluate the effect of the 5-LOX inhibitor on osteoclast activity using an LPS-induced periodontal disease model.	5-LOX inhibitor reduced the level of inflammatory mediators in gingival tissues and modulated osteoclast activity.

Abbreviations. 5-HETE: 5-hydroxyeicosatetraenoic acid; 5-LOX: 5-lipoxygenase; BMM: bone marrow derived macrophages; CysLTR: cysteinyl leukotriene receptor.

Table 2. Effect of 5-LOX metabolites on the osteoblast function

Author and Year	Study design	Animals/cell-line used	Methodology description	Study outcome
Ren W 1991 [34]	In-vitro	Rat calvaria osteoblast cells, Saos-2 and G292 cell-line	To investigate the effect of LTB4 and LTC4 on the osteoblast activity.	LTB4 inhibits osteoblast DNA synthesis, thereby suggesting the detrimental effect of 5-LOX metabolites on bone health.
Traianedes K 1998 [49]	In-vitro	Rat and mice calvaria cells	To investigate the role of dexamethasone, rhBMP-2, 5-HETE, LTB4, and LTD4 on bone nodule formation, ALP, and cell proliferation.	Dexamethasone and rhBMP-2 improved bone formation, while 5-HETE and LTB4 prevented their action and reduced ALP activity.

Abbreviations. 5-HETE: 5-hydroxyeicosatetraenoic acid; 5-LOX: 5-lipoxygenase; ALP: alkaline phosphatase; LTB4: leukotriene B4; LTC4: leukotriene C4; LTD4: leukotriene D4; rhBMP-2: recombinant human bone morphogenetic protein-2.

Table 3. Effect of 5-LOX on bone formation

Author and Year	Study design	Animals/cell-line used	Methodology description	Study outcome
Manigrasso MB 2010 [50]	In-vivo	5-LOX and COX knockout C57BL/6 mice	To compare the femur fracture healing rate in 5-LOX knockout mice compared to control mice.	5-LOX knockout mice exhibited accelerated healing with improved mechanical properties; COX knockout mice had significantly higher leukotriene levels which suggests that the arachidonic acid pathway was shifted towards the 5-LOX pathway in the absence of COX.
Wixted JJ 2009 [51]	In-vivo	C57BL/6 mice	To evaluate the effect of 5-LOX and CysLTR inhibitors on the rate of femur fracture healing time.	5-LOX and CysLTR inhibitors improved fracture repair with increased callus size and early bone formation. Gene expression of bone-formation-related genes like *Col 2a1*, *Sox9*, *Runx2*, *Col X*, and Osteocalcin was increased.
Cottrell JA 2013 [53]	In-vivo	Sprague-Dawley rats	To investigate the effect of a 5-LOX inhibitor-loaded scaffold on the rate of bone formation in an induced-femur defect animal model.	5-LOX inhibitor increased the bone formation rate in a dose-dependent manner.
Cottrell JA 2009 [52]	In-vivo	Sprague-Dawley rats	To investigate the effect of COX and 5-LOX inhibitors on femur fracture healing rate.	Bone fracture healing rate was significantly delayed by COX inhibitors, while it was significantly improved by 5-LOX inhibitor treatment.
Biguetti CC 2020 [54]	In-vivo	Wild-type and 5-LOX knockout mice	To compare the bone fracture healing rate in 5-LOX knockout mice and control wild-type mice.	5-LOX knockout mice showed an improved bone healing rate compared to wild-type mice. This action was attributed to increased *Runx2* gene expression.
Saul D 2021 [55]	In-vivo	OVX and normal SD rats	To evaluate the effect of 5-LOX inhibitors on bone health parameters.	Treatment of 5-LOX inhibitors enhanced callus formation and showed a positive effect on bone formation rate.
Saul D 2019 [56]	In-vivo	OVX and normal SD rats	To evaluate the effect of 5-LOX inhibitor on bone health parameters.	5-LOX inhibitor treatment enhanced tibial callus density and cortical width suggesting a positive impact of 5-LOX inhibitor on the bone repair process.
Lee JM 2012 [44]	In-vivo	ICR mice	To evaluate the effect of 5-LOX inhibitor on LPS-induced osteoclast activity and bone health.	5-LOX inhibitor treatment markedly reduced LPS-induced bone resorption and improved bone mineral density.

Abbreviations. 5-LOX: 5-lipoxygenase; COX: cyclooxygenase; CysLTR: cysteinyl leukotriene receptor; LPS: lipopolysaccharide; OVX: ovariectomized.

Conclusion

Bone fracture healing is a complex and harmonized process involving various steps, in which the development of a favorable inflammatory environment plays a pivotal role. While inflammation is a result of various inflammatory cytokines, the role of 5-LOX and its metabolites is estimated to be beyond the inflammatory phase. By acting on gene expression level, 5-LOX metabolites have a crucial role in altering the normal activity of osteoclasts and osteoblasts and subsequent bone formation and bone healing rate. While the current treatment for bone fracture healing includes various options, the therapies targeting the 5-LOX cycle are largely unexplored. The current review was conducted to highlight the potential of 5-LOX targeted therapies for improving the bone fracture healing process, and the results of the current review might provide a roadmap for future large-scale pre-clinical research and clinical studies as well.

Conflicts of interest

All authors declare that they are employees of Sundyota Numandis Probioceuticals Pvt. Ltd. (Ahmedabad, Gujarat, India). All authors declare no other competing interests.

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