

Licofelone: A Novel Non-Steroidal Anti-Inflammatory Drug (NSAID) in Arthritis

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ABSTRACT

Arthritis refers to different medical conditions associated with disorder of the primary structures that determine joint functions such as bones, cartilage and synovial membranes. Drug discovery and delivery to retard the degeneration of joint tissues are challenging. Current treatments of different arthritis involves administration of ideal non-steroidal anti-inflammatory drugs (NSAIDs) but are frequently associated with adverse reactions, related to inhibition of cyclo-oxygenase (COX) in tissues where prostaglandins exert physiological effects, such as gastric mucosal defense and renal homeostasis. As a consequence, the interest for novel approaches has re-emerged. New therapeutic options, still under clinical evaluation, are represented as dual COX and 5-lipoxygenase (5-LOX) inhibitors. These are expected to possess clinical advantages over the selective inhibitors of COX enzyme. One of the most promising compounds belonging to this category, licofelone, a competitive inhibitor of 5-lipoxygenase, cyclooxygenase (COX-1 and COX-2), is currently in clinical development for the treatment of osteoarthritis (OA). Licofelone decreases the production of pro-inflammatory leukotrienes and prostaglandins which are involved in the pathophysiology of OA and in gastrointestinal (GI) damage induced by NSAIDs and have the potential to combine good analgesic and anti-inflammatory effects with excellent GI tolerability. The emerging clinical data for licofelone indicate that it is an effective and well-tolerated therapy which could be suitable for the long-term treatment of patients with OA. This review focuses upon the gastrointestinal (GI) safety of selective COX-2 inhibitors and of novel therapeutic strategies, in comparison with traditional NSAIDs.

Keywords: *Review, Licofelone, Safety, NSAIDs, Cyclo-oxygenase inhibitor, Lipoxygenase inhibitor*

The human skeleton and muscle help to make possible the peaceful gyration as well as more safe routine movements of everyday life. Our body naturally produces glucosamine, which is used to produce proteoglycans, the principal lubricating proteins in our cartilage, tendons, ligaments, synovial fluid and mucous membranes. Imbalance in the regeneration process of these functional elements of the joint leads to friction, pain and inflammation, due to cartilage repair decreases/ceases, cartilage become thinner and bones grind against one another. Arthritis and many other rheumatic diseases are mainly disorders of the supportive or connective tissues of the bones, tendons, heart valves and joints ranging from minor stiffness to engrave disability and deformity [01]. Although many specific drugs for treatment of major inflammation and/or acute pain like steroidal anti-inflammatory agents or narcotic analgesics are available but ideal NSAIDs should affect only controlled inflammation by

modifying inflammatory response to diseases, but not to interfere with normal inflammatory process. NSAIDs effectively quell the pain and reduce the inflammation that can occur in advanced cases, but they do nothing to halt the disease process [2]. As awareness of the GI side effects associated with NSAIDs increases, safety becomes a primary requisite in treatment. Numerous articles examining the gastric and duodenal damage caused by NSAIDs have been published, however, only recently have the more distal intestinal disturbances induced by these drugs received close attention. There is often a poor correlation between patient reported symptoms of upper GI distress and endoscopically-proven gastropathy. This may suggest afflictions of more distal parts of the intestine. The concept of selective and site-specific damage to the upper GI tract following NSAIDs has been questioned, particularly by the works of Bjarnason, who has demonstrated that patients on chronic NSAID therapy can develop small



Fig 1. Anatomy of knee joint

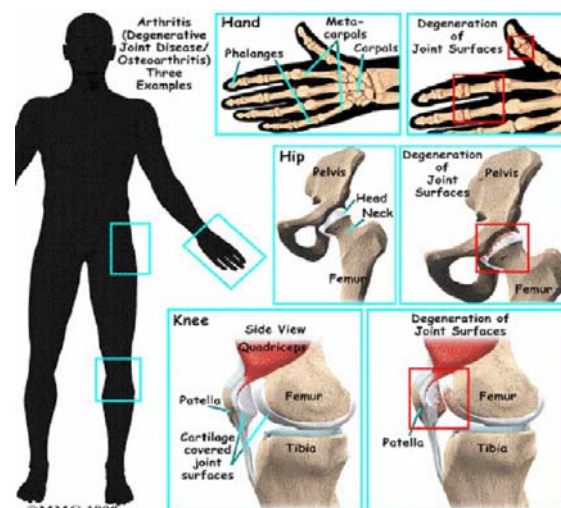


Fig 2. Arthritis in various joints

and large intestinal inflammation which may lead to anaemia, hypoalbuminemia, ulceration, 'diaphragm' like strictures, perforation, and hemorrhage [3].

Medical researchers had developed a number of NSAIDs that blocked the body's production of substances playing vital roles in pain and inflammation responses. Osteoarthritis (OA) is a complex disease that is characterized by joint pain, inflammation and stiffness caused by the degradation of collagen and proteoglycans in cartilage tissue. Although the exact cause or trigger for OA is not clear, many mediators are known to be involved in the pathophysiology and progression of the disease. These include cartilage-degrading enzymes, cytokines and, as reviewed below, leukotrienes (LTs) and prostaglandins (PGs), which contribute to pain and inflammation in OA. Blocking the cyclooxygenase (COX) pathway of arachidonic acid (AC) metabolism by conventional non-steroidal anti-inflammatory drugs (NSAIDs) not only results in decreased production of gastroprotective prostaglandins (PGs) but also in the increased metabolism of AC via the 5-lipoxygenase (5-LOX) route. This 'shunting effect' leads to increased production of leukotrienes (LTs), which contribute to inflammatory processes and further gastrointestinal (GI) damage. Thus, developing dual COX/5-LOX inhibitors may enhance anti-inflammatory effects and reduce the undesirable side effects associated with NSAIDs, especially those in the GI tract [4]. Licofelone is the most promising of the dual COX/5-LOX inhibitors discovered by Merckle GmbH and is being developed by Euro Alliance (a consortium of Alfa Wassermann SpA, Lacer SA and Merckle). Pharmacological studies have demonstrated that the drug has analgesic, antipyretic, anti-inflammatory and significant anti-asthmatic activity without causing GI damage. It is currently in a phase III trial for the treatment of osteoarthritis (OA) [5].

What Is Arthritis?

Arthritis is a term that includes a group of disorders that affect your joints and muscles. Arthritis symptoms

include joint pain, inflammation and limited movement of joints. When a joint is inflamed, it may be swollen, tender, warm to the touch or red. Surrounding each joint is a protective capsule holding a lubricating fluid to aid in motion (Fig 1). Cartilage, a slippery smooth substance, covers most joints to assure an even, fluid motion of the joint. With joint arthritis, the cartilage may be damaged, narrowed and lost by a degenerative process or by inflammation making movement painful (Fig 2). For most people arthritis pain and inflammation cannot be avoided as the body ages. In fact, most people over the age of 50 show some signs of arthritis. Joints naturally degenerate over time. Fortunately, arthritis can be managed through a combination of medication, exercise, rest, weight-management, nutrition, and, in some cases, surgery. Arthritis is not just one disease; it is a complex disorder that comprises more than 100 distinct conditions and can affect people at any stage of life. Two of the most common forms are osteoarthritis and rheumatoid arthritis [6].

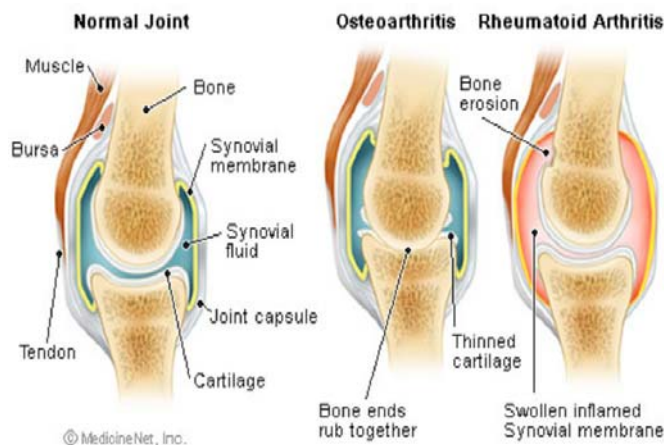
Types of Arthritis

Primary forms of arthritis

1. Osteoarthritis.
2. Rheumatoid arthritis
3. Septic arthritis
4. Gout and pseudogout
5. Juvenile idiopathic arthritis
6. Ankylosing Spondylitis

Secondary to other Diseases

1. Lupus erythematosus
2. Sarcoidosis
3. Henoch-Schonlein purpura
4. Psoriatic arthritis
5. Reactive arthritis
6. Haemochromatosis
7. Hepatitis
8. Wegener's granulomatosis.
9. Lyme disease
10. Familial Mediterranean fever



Normal and Arthritic Joints

Fig 3. Normal and arthritic joints

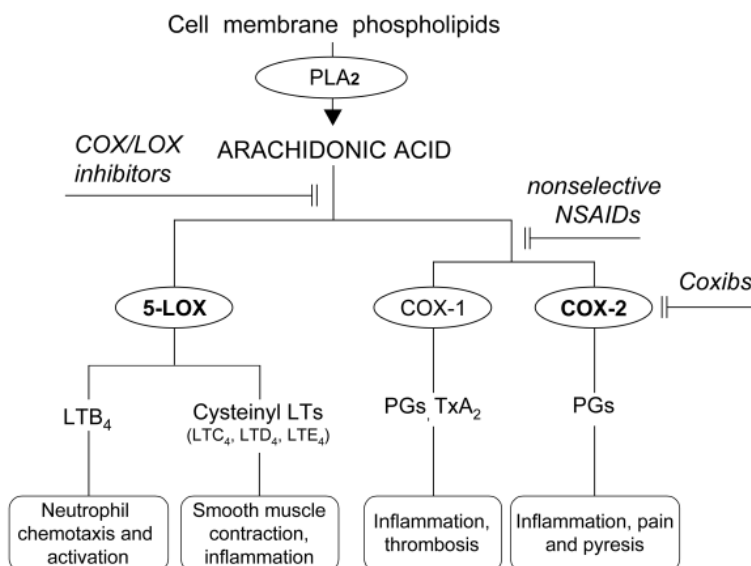


Fig 4. General scheme representing the main metabolic pathways leading to arachidonic acid products involved in the inflammatory process. Targets of anti-inflammatory drugs are also shown. COX = cyclooxygenase; 5-LOX = 5-lipoxygenase; LTs = leukotrienes; PGs = prostaglandins; PLA₂ = phospholipase A₂; TxA₂ = thromboxane A₂.

- 11. Hyperimmunoglobulinemia D with recurrent fever.
- 12. TNF receptor associated periodic syndrome.
- 13. Inflammatory bowel disease (Including Crohn's Disease and Ulcerative Colitis)

Osteoarthritis

Osteoarthritis (OA, also known as degenerative arthritis degenerative joint disease), is a group of diseases and mechanical abnormalities entailing degradation of joints i.e. articular cartilage and next to the subchondral bone (Fig 3). Clinical symptoms of OA may include joint pain, tenderness, and also stiffness, inflammation, creaking, and locking of joints. In OA, a variety of potential forces; hereditary, developmental, metabolic, and mechanical may initiate processes leading to loss of cartilage; a strong protein matrix that lubricates and cushions the joints. As the body struggles

to contain ongoing damage, immune and re-growth processes can accelerate damage. When bone surfaces become less well protected by cartilage, subchondral bone may be exposed and damaged, with re-growth leading to a proliferation of ivory-like, dense, reactive bone in central areas of cartilage loss, a process is called eburnation. The patient increasingly experiences pain upon weight bearing, including walking and standing. Due to decreased movement because of the pain, regional muscles may atrophy and ligaments may become more lax [7,8].

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic, systemic anti-inflammatory disorder that may affect many tissues and organs, but principally attacks the joints producing an inflammatory synovitis that often progresses to

destruction of the articular cartilage and ankylosis of the joints (Fig 3). Rheumatoid arthritis can also produce diffuse inflammation in the lungs, pericardium pleura and sclera and also nodular lesions, most common in subcutaneous tissue under the skin. Although the cause of rheumatoid arthritis is unknown, autoimmunity plays a pivotal role in its chronicity and progression. About 1% of the world's population is afflicted by rheumatoid arthritis, women three times more often than men. Onset is most frequent between the ages of 40 and 50, but no age is immune. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility [8].

Nonsteroidal anti-Inflammatory Drugs (Nsaids)

NSAIDs are among the most-commonly prescribed categories of drugs worldwide in the treatment of pain and inflammation in many conditions. Prostaglandins (PGs) are an important group of chemical mediators which are responsible for producing changes, symptoms and signs of inflammation. NSAIDs are heterogeneous group of compounds that are non-selectively by inhibiting both cyclooxygenase enzymes (COX-1 and COX-2) which are responsible for the biosynthesis of the prostaglandins (PGs) and thromboxanes from its precursor arachidonic acid. COX-1 enzyme is constitutively present in cells. It is a house-keeping enzyme has a physiological function producing prostaglandins which help to keep the stomach and blood vessels clean by making prostacyclin, whereas COX-2 is inducible form and is generated at sites of inflammation. It is reported that NSAIDs provides pain relief by reducing prostaglandin, bradykinins and oxygen radicals. Non selective COX inhibitors produces side effects on long term use and to overcome this problem, selective COX-2 inhibitors were developed. COX-2 inhibitors decreases COX-2 mRNA levels and modulates local and systemic cytokine production in order to reduce inflammation and bone erosion [9,10].

Classification of NSAIDs

A) Non-Selective Cox-Inhibitors

1. Salicylates:-Aspirin, Difunisal
2. Pyrazolone derivatives: - Phnylbutazone, Oxyphenylbutozone
3. Indole derivatives: -Indomethacin, Etodolac, Sulindac
4. Propionic acid derivatives: -Ibuprofen, Ketoprofen, Flurbiprofen, Dexibuprofen, Naproxen, Fenoprofen, Indoprofen, Benoxaprofen, Pirprofen
5. Anthranilic acid derivatives: - Mefenamic, Meclofenamic, Niflumic
6. Aryl-acetic acid derivatives: - Diclofenac, Aceclofenac, Fenclofenac
7. Oxicam derivatives: -Piroxicam, Tenoxicam, Sudoxicam, Isoxicam, Meloxicam
8. Pyrrolo-pyrrole derivatives: -Ketorolac

B) Preferential Cox-2 Inhibitors

Nimesulide, Meloxicam, Nabumetone.

C) Selective COX-2 Inhibitors

Celecoxib, Rofecoxib, Valdecoxib.

D) Analgesic-Antipyretics

Paracetamol, Metamizol, Propiphenazone, Nefopam.

E) LOX-5 Inhibitors

Licofelone

Mechanism of NSAIDs

The mechanism of action of NSAIDs is the inhibition of the enzyme cyclooxygenase, which catalyzes arachidonic acid to prostaglandins and leukotrienes. Arachidonic acid is released from membrane phospholipids as a response to inflammatory stimuli. Prostaglandins establish the inflammatory response.

The Cyclooxygenase Pathway

NSAIDs work by interfering with the cyclooxygenase pathway. Different mechanisms stimulate the two different types of cyclooxygenase. COX-1 is stimulated continuously by normal body physiology. The COX-1 enzyme is constitutive, meaning that its concentration in the body remains stable. It is present in most tissues and converts arachidonic acid to prostaglandins. These prostaglandins in turn stimulate body functions, such as stomach mucous production and kidney water excretion, as well as platelet formation. The location of the COX-1 enzyme dictates the functions of the prostaglandins it releases. For example, COX-1 in the stomach wall produces prostaglandins that stimulate mucous production. In contrast, the COX-2 enzyme is induced. It is not normally present in cells but its expression can be increased dramatically by the action of macrophages, the scavenger cells of the immune system. COX-2 plays a very important role in inflammation. COX-2 is involved in producing prostaglandins for an inflammatory response. COX-1 is stimulated continually, and COX-2 is stimulated only as a part of an immune response [11].

Inflammatory Role of Prostaglandin

Prostaglandins are paracrine secretions (local hormones) - they are released from cells and bring about changes in neighboring cells that carry specific prostaglandin receptors in their membranes. They are rapidly degraded locally, and generally do not reach the blood stream. The influence, which prostaglandins have, depends upon the type of tissue they are acting upon. Such action may be direct, or as a result of modifying the actions of other signaling molecules. Prostaglandins were released by damaged cells and nearby macrophages and one of their effects to stimulate pain receptors (nociceptors). At the same time they intensify the effects of other chemical mediators such as histamine and bradykinin. Acting in concert these substances can bring about vasodilatation and an increase in the permeability of capillaries supplying the damaged area, encouraging the migration of phagocytes from the blood through capillary walls into the damaged tissue. As a result of these changes, the blood supply to

the area increases, the tissues swell, and pain occurs, signs of inflammation [12].

Action of NSAIDs on Cyclooxygenase

The two forms of cyclooxygenase have equal molecular weights and are very similar in structure. However, the attachment site of COX-1 is smaller than the attachment site of COX-2. Therefore, it accepts a narrower range of structures as substrates. The cyclooxygenase active site lies at the end of a long, narrow, hydrophobic tunnel or channel. Three of the alpha helices of the membrane-binding domain lie at the entrance to this tunnel. In various ways, they all act by filling and blocking the tunnel, preventing the migration of arachidonic acid to the active site at the back of the tunnel. They do this by temporarily blocking the attachment site for arachidonic acid on the cyclooxygenase enzyme, thereby preventing it from converting arachidonic acid to prostaglandin. The exception is aspirin, which irreversibly acetylates cyclooxygenase. It takes longer for the effects of aspirin to wear off because new enzymes must be formed by the body to replace the altered enzymes. When COX-1 is acetylated by aspirin, the site for arachidonic acid is blocked. However, when aspirin acetylates COX-2, the active site is still large enough to accept arachidonic acid [13,14].

Cyclooxygenase Inhibition and Gastrointestinal Damage

Non-selective non-steroidal anti-inflammatory drugs (NSAIDs), such as naproxen, inhibit both COX-1 and COX-2 in the AA pathway and are commonly used in the treatment of OA. This leads to a reduction not only in the levels of the PGs (largely produced by COX-2) that cause pain and inflammation in OA, but also in the levels of gastroprotective PGs (mainly produced by COX-1). The resulting GI damage has been well documented as a serious complication associated with the use of NSAIDs, as discussed in the previous article in this Supplement. Selective COX-2 inhibitors, such as celecoxib and rofecoxib, were developed to overcome the GI disadvantages of NSAIDs and are also used to treat the signs and symptoms of OA. These agents reduce the levels of inflammatory PGs while leaving the levels of gastroprotective PGs largely unaltered [15-17]. However, it has emerged that there is still a risk of GI damage associated with the use of selective COX-2 inhibitors. Accumulating evidence suggests that this may be because of an overlap in the roles of COX-1 and COX-2 in physiological and pathophysiological processes. One trial comparing a selective COX-2 inhibitor (rofecoxib) with a non-selective NSAID (naproxen) found that the incidences of confirmed GI events were 2.1 and 4.5 per 100 patient-years, respectively, leading to a relative risk of 0.5 ($p < 0.001$) [18]. However, the risk of GI complications with selective COX-2 inhibitor therapy is greater in patients who are elderly, taking low-dose aspirin or have a history of GI ulceration [19]. Selective COX-2 inhibitors may also delay GI ulcer healing, possibly by

shifting the balance of angiogenic factors to reduce new blood vessel growth [20]. Complications arising from GI ulcers can prove fatal, and therefore inhibition of ulcer healing is an undesirable side-effect, particularly in patients with an increased risk of developing ulcers. It has been proposed that the inhibition of one or both COX enzymes, while reducing the levels of gastrotoxic PGs, may result in alternative processing of AA via the 5-LOX pathway. This increases the production of cysteinyl LTs and LTB₄, which contribute to GI toxicity by promoting the migration of leucocytes, breaking down the mucosal barrier and stimulating gastric acid secretion. One study examined gastric mucosal synthesis of LTB₄ in patients taking NSAIDs and found the median level of LTB₄ was 0.9 pg/mg (0.2–2.5 pg/mg), compared with 0.0 pg/mg (0.0–0.6 pg/mg) in patients not taking NSAIDs ($P < 0.001$). The increased levels of LTB₄ were associated with GI damage [21].

PGs have a role in renal function and vascular homeostasis. It is perhaps not surprising, therefore, that the modulation of PG levels through the activity of selective COX-2 inhibitors and nonselective NSAIDs is associated with adverse renal effects such as fluid and electrolyte disturbances and blood pressure elevation [22–23]. Moreover, selective COX-2 inhibitors may interfere with blood pressure control in hypertension via variable mechanisms.

Furthermore, selective COX-2 inhibitors have been associated with thromboembolic complications such as myocardial infarction. Consequently, selective COX-2 inhibitors should be used with caution in patients with a history of, or at risk of, cardiovascular or renal disease. In addition to the role of LTs and PGs in GI damage/protection, there is evidence to suggest that the adhesion of leucocytes to mesenteric venules could play a role in the development of NSAID induced GI damage [24,25]. This could be caused by leucocyte chemotaxis due to increased LT levels following a shunt in AA metabolism to the 5-LOX pathway. A study was conducted to compare the adherence of leucocytes to mesenteric venules in rats treated with indomethacin or licofelone [26]. Within 60 min of indomethacin administration, there was a substantial increase in leucocyte adhesion. This increase continued over the course of the study (120 min), and was statistically significant when compared with the control ($p < 0.05$). Conversely, licofelone was associated with only a small increase in adhesion, which did not increase further after 90 min.

Role of Leukotrienes and Prostaglandins in Osteoarthritis

LTs and PGs are produced by the activity of three enzymes, namely 5-lipoxygenase (5-LOX), cyclooxygenase (COX-1 and COX-2), as part of the arachidonic acid (AA) pathway (Fig 4) [1–3]. 5-LOX (together with other enzymes) converts AA to the leukotrienes LTB₄, LTC₄, LTD₄ and LTE₄. COX-1 converts AA to, among other molecules, thromboxanes, such as TXA₂, and PGs, such as PGD₂, PGE₂, PGF₂ and

PGI₂ (prostacyclin). The activity of COX-2 leads to production of a narrower spectrum of PGs, specifically PGE₂ and PGI₂. The AA pathway, its products and the enzymes mediating their formation play a major role in many aspects of human physiology, including vascular homeostasis, gastroprotection, renal homeostasis and bone formation, and pathophysiological processes, including pain and inflammation in OA [27,28]. PGs have various physiological and pathophysiological effects. For example, PGI₂ and PGE₂ affect vascular homeostasis because they have potent effects on vasodilation and vascular permeability, and PGI₂ also inhibits platelet aggregation. Furthermore, both PGI₂ and PGE₂ are involved in modulating normal glomerular filtration rate and blood flow. The vasodilatory properties of these two molecules increase mucus production and reduce acid and pepsin levels in the stomach, thereby protecting the integrity of the gastrointestinal (GI) mucosa. Of particular relevance to OA is the observation that increases in PGI₂ and PGE₂ levels result in hyperalgesia, leading to pain, and also inflammation [29]. Additionally, PGs in particular PGE₂ are thought to stimulate bone resorption by increasing the number of osteoclasts, which could contribute to the joint damage seen in OA. Many of the processes affected by PGs are also affected by LTs, including vascular homeostasis, vascular permeability, gastric vessel constriction and gastric acid secretion [30]. For example, the cysteinyl LTs (LTC₄, LTD₄ and LTE₄) are vasoconstrictors and play an important role in mediating vascular permeability. Their vasoconstrictive effects are antagonistic to the effects of PGs and result in gastric vessel vasoconstriction, gastric acid secretion, pro-inflammatory cytokine production and consequent GI damage [31]. In addition, the cysteinyl LTs have powerful effects on the smooth muscle of the airways, leading to bronchoconstriction. LTB₄ and the cysteinyl LTs are potent mediators of inflammation, causing increased activation, recruitment, migration and adhesion of immune cells. Indeed, the levels of LTB₄ and PGE₂ in particular are higher in the joints of patients with OA than in those of healthy individuals [32,33]. Furthermore, LTB₄ increases the production and release of the cytokines, tumour necrosis factor- α (TNF- α) and interleukin-1- β (IL-1 β). This is important in OA because these cytokines are thought to mediate damage to cartilage by increasing the expression of degradative enzymes and reducing the repair of damaged cartilage by chondrocytes. Similarly to PGE₂, LTB₄ is also thought to have a role in stimulating bone resorption in OA, and this may be owing to increased TNF- α and IL-1 β production [34]. Although many of these observations are from *in vitro* studies, it is likely that they are pertinent, at least in part, to human OA. These molecules have a range of physiological functions. LXA₄ and LXB₄ are formed as part of the inflammatory response, but, in contrast to LTs and PGs, have anti-inflammatory effects and inhibit LT-stimulated chemotaxis (Fig 5). Endocannabinoids

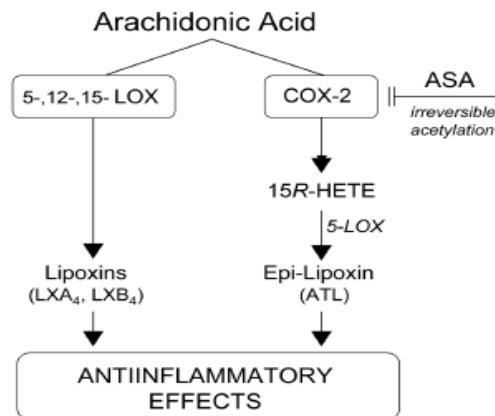


Fig 5. Synthesis of anti-inflammatory lipoxins (LXs) from arachidonic acid. Irreversible acetylation of COX-2 enzyme by aspirin leads to the formation of a lipoxin epimer (Epi-lipoxin), also known as aspirin-triggered lipoxin (ATL) endowed with anti-inflammatory properties. 15R-HETE = 15Rhydroxyeicosatetraenoic acid; LOX = lipo-oxygenase.

are thought to function as neuromodulators and vasodilators [35] and also as mediators of antinociception [36].

Dual COX/5-LOX Inhibitors

In recent years it has been clarified that several mediators of the arachidonic acid metabolism are involved in the inflammatory process. Leukotrienes (LTs), which are the second main family of arachidonate products, are synthesized from the activity of 5-lipoxygenase (5LOX) and have a major role in the inflammatory response. LTs are extremely potent vasoactive and leucotactic compounds, that are in some respects more inflammogenic than PGs. LTB₄, in particular, induces recruitment of leukocytes to inflamed sites, lysosomal release in neutrophils, adhesion molecule expression and subsequent plasma leakage [37,38]. This finding has suggested that dual inhibition of both LTs and PGs may lead to enhanced and wider anti-inflammatory activity. Moreover, it can also be expected that combined COX and LOX inhibition may originate an improved GI safety profile, due to a number of adverse effects of LTs in the GI mucosa, which impair mucosal integrity and exacerbate the damaging effect of noxious stimuli [39]. In particular, reduction of mucosal blood flow, leukocyte-endothelial cell interaction and leukocyte infiltration are considered a prerequisite for NSAID-induced gastropathy. In line with this, several studies have demonstrated that 5-LOX inhibitors or LT receptor antagonists exert protective effects on acute and chronic gastric mucosal damage in various ulcer models, including NSAID-induced gastric lesions [40]. These observations, along with the possibility that COX inhibition by NSAIDs can divert arachidonate to lipoxygenase pathway, led to the theory that excess LT production, combined with PG deficit, could contribute to NSAID-induced mucosal damage [41]. In line with this, elevated production of LTB₄ by the human

stomach has been documented in patients taking NSAIDs [42]. One compound in the 5-LOX/COX series of NSAIDs is licofelone (previously named ML3000), which in animal experiments and in clinical trials showed anti-inflammatory effects comparable to conventional NSAIDs, but with an improved GI safety profile [43]. In conclusion, results with dual COX/5-LOX inhibitors seem to be promising; however, although the dual inhibition concept appears a rather logical approach, the lesson from COX-2 inhibitors demand to be cautious before drawing definite conclusions about the pharmacological profile of a new class of drugs. Large clinical trials will establish in the future whether theoretical expectations on safety and efficacy of these drugs are achieved.

Licofelone

Licofelone ML3000 2-[6-(4-chlorophenyl)-2,2-dimethyl-7-phenyl-2,3-dihydro-1H-pyrrolizin-5-yl] acetic acid. Licofelone is the most promising of the dual COX/5-LOX inhibitors discovered by Merckle GmbH and is being developed by EuroAlliance (a consortium of Alfa Wassermann SpA, Lacer SA and Merckle). Pharmacological studies have demonstrated that the drug has analgesic, antipyretic, anti-inflammatory and significant anti-asthmatic activity without causing GI damage. It is currently in a phase III trial for the treatment of osteoarthritis (OA) [44,45]. Licofelone decreases the production of both LTs and PGs, and thereby reduces inflammation and pain with low gastrotoxicity. Licofelone (ML3000) is a dual inhibitor of cyclooxygenases (COX-1 and COX-2) and 5-lipoxygenase (5-LOX). Anti-inflammatory drugs with this mode of action are considered as attractive therapeutics for the treatment of arthritic diseases [46,47]. Almost three decades ago, the very first COX-2/5-LOX-inhibitor benoxaprofen entered the market and, at that time, compared to the conventional non-steroidal anti-inflammatory drugs (NSAIDs), this compound not only showed equivalent efficacy but also an advantageous gastro-intestinal safety profile. Unfortunately, this compound was hepatotoxic which led to its withdrawal from the market [48]. In the late 80s, several companies were running drug-discovery programs to develop dual COX/LOX inhibitors as NSAIDs with an improved gastro-intestinal safety. Most projects were terminated after the discovery of the inducible COX-2 and its identification as that isoform which is directly linked with the inflammatory process. After demonstrating advantageous gastro-intestinal safety in a series of clinical trials, COX-2-selective drugs (coxibs) achieved blockbuster-drug status very soon after entering the market. Today, it is known that all coxibs are burdened with a very low but unequivocal risk to induce life-threatening or fatal. Apart from the first clinical experience with benoxaprofen, there is a lot of non-clinical and clinical evidence, that dual inhibition of the COX and 5-LOX pathways combine the advantages of conventional NSAIDs and coxibs, i.e. good anti-inflammatory, analgesic activity and gastro-intestinal as well as cardiovascular safety. As a promising candidate,

licofelone (ML3000) has been developed for treatment of arthritic conditions. The mechanism of action of licofelone, i.e. inhibition of both COX-1/-2 and 5-LOX, was demonstrated in pharmacological studies as well as in experimental disease models [49].

Initial synthesis of licofelone proceeded with poor overall yield (< 5%) [50]. Cossy and Belotti reported a short and efficient synthesis of licofelone that featured a thermal-acidpromoted bicyclization of an ω -acetylenic amino ester [51]. Basic phase-transfer catalysis of 1-chloro-3-phenyl-2-propyne with isobutyraldehyde in the presence of NaI produced an aldehyde. This aldehyde was condensed with methyl glycinate hydrochloride under reductive amination conditions to yield an ω -acetylenic amino ester, which was heated at 150°C in the presence of 1 equivalent of pivalic acid to give tetrahydro-6-oxo-1H-pyrrolizine. Acylation with diethyl oxalate under basic conditions introduced a carboxylic acid side chain. The resulting β -diketone was entirely enolized. A Suzuki cross-coupling reaction was used to introduce the 4-chlorophenyl group. Treatment with Nphenyltrifluoromethanesulfonimide produced a triflate. This was coupled with (4-chlorophenyl) boronic acid in the presence of Pd(PPh₃)₄ and Na₂CO₃/H₂O in refluxing tetrahydrofuran. The ethyl ester of licofelone was produced by deoxygenation via the 4-toluenesulfonylhydrazone, which after saponification led to licofelone at an overall yield of 19%. From a series of 6,7-diaryldihydropyrrolizin-5-yl acetic acids, licofelone, with its 4-chloro substituent, was the most potent and well-balanced dual inhibitor of both COX and 5-LOX [51].

Pharmacology

The inhibition of COX and 5-LOX by licofelone was first determined in a bovine thrombocyte intact cell assay and intact bovine polymorphonuclear leukocytes, respectively

(IC₅₀ values of 0.21 μ M for COX and 0.18 μ M for 5-LOX) [52]. In a human whole blood assay, licofelone (0.3, 1.0, 3.0, 10 and 30 μ g/ml) and indomethacin (0.3, 1.0, 3.0, 10 and 30 μ g/ml) concentration-dependently inhibited the synthesis of PGE₂ (IC₅₀ = 3.9 and 4.5 μ M, respectively). In contrast to licofelone, indomethacin produced an increase in LTC₄ of up to 155.5% of control. Furthermore, licofelone (1 to 10 μ M) inhibited the synthesis of LTB₄ in a concentration related manner (IC₅₀ = 3.6 μ M) in a basophilic leukemia cell assay using RBL-1 cells [53]. Licofelone inhibited LTC₄ formation by mixed polymorphonuclear leukocyte/platelet suspensions stimulated with A-23187 (IC₅₀ = 3.8 μ M). Licofelone also inhibited the generation of reactive oxygen species, release of elastase by polymorphonuclear leukocytes, and homotypic polymorphonuclear leukocyte aggregation induced by N-formyl-methionyl-leucyl-phenylalanine (fMLP), complement fraction 5a (C5A) and platelet activating factor (PAF), respectively [54]. These in vitro studies demonstrated that licofelone inhibits 5-LOX as well as COX-1 and COX-2 activity, and therefore, polymorphonuclear leukocyte responses relevant to the

pathogenesis of inflammation. The pharmacodynamic profile of licofelone has been assessed and compared with widely used NSAIDs in various animal models. In a carrageenan-induced rat paw edema model, licofelone (10, 30 and 100 mg/kg) demonstrated an ED₅₀ value of 17 mg/kg po and completely inhibited both PGE₂ and LTB₄ secretion, compared with indomethacin (10 mg/kg), which only inhibited secretion of PGE₂ [55].

An oral dose of licofelone (10 mg/kg) was more effective than an oral dose of aspirin (50 mg/kg) in a mouse phenylquinone writhing model [56]. The Randall and Selitto assay demonstrated that licofelone (30 mg/kg) was as effective as indomethacin (10 mg/kg) at increasing the pain threshold of inflamed paws in rats [56]. In vitro, licofelone (0.8 to 8 μM) inhibited the production of PGE₂ and LTB₄ by OA osteoblasts at the highest dose, dose-dependently stimulated 1,25-dihydroxy vitamin D-induced alkaline phosphatase activity, and inhibited osteocalcin release via its effect on LTB₄ production [57]. These results suggest that licofelone could be used as a disease-modifying drug for the treatment of OA and rheumatoid arthritis (RA). The gastric-sparing properties of licofelone have also been investigated. The drug dose-dependently inhibited ATPase activity in pig gastric microsomes with an IC₅₀ value of 16.6 μM. When the drug was diluted by 100-fold, the inhibitory effect was abolished. Licofelone-treated human gastric adenocarcinoma cells secreted less baseline and IL-1β-induced IL-8 with IC₅₀ values of 0.82 and 1.2 μM, respectively [57].

Metabolism

Plasma levels and distribution of radioactivity were examined using whole-body autoradiography after oral administration of ¹⁴C-labeled licofelone (13.7 to 26.6 mg/kg) to female rats. Plasma levels of licofelone peaked at 3 to 4 h after administration, with a plasma t_{1/2} of ~ 11 h. The highest tissue levels of licofelone were detected in the lung, liver, kidney, heart and intestine. Almost no penetration of the blood-brain barrier was noted; however, after 48 h there was a minor accumulation in fat. Of the total radioactivity, 58.3% was found in the feces and 7.9% in the urine [58].

Licofelone (200 mg bid for 5 days and a single final dose of 200 mg on day 6) was administered in 18 healthy male and female young (mean age of 30.9 years) and elderly (mean age of 72.1 years) individuals. Following the first dose, mean C_{max} was similar for young (1665 ± 1151 ng/ml) and elderly (1637 ± 903 ng/ml) individuals. The maximum plasma concentrations were reached 0.74 to 4 h after administration, while the mean AUC(0 to 12) was 23% lower in the young individuals (5646 ± 2073 versus 4582 ± 1927 ng.h/ml). Licofelone demonstrated similar C_{max} values in the two groups at steady-state, with young individuals having a C_{max} value of 1727 ± 829 ng/ml and elderly individuals having a C_{max} value of 1744 ± 616 ng/ml; the AUC was 20% higher in elderly individuals. The t_{1/2} (β) was greater in young individuals than elderly ones (11.1 ± 7.0 versus 8.7 ± 4.7 h), while the mean t_{1/2} (α) value was 15% higher in the elderly

study population [47]. No pharmacokinetic interaction between licofelone and warfarin was observed, suggesting that the two drugs have different elimination pathways [58].

Evidence of Gastrointestinal Safety with Licofelone

The GI tolerability of licofelone compared with NSAIDs has been investigated in a rat model by examining the levels of the gastroprotective, proinflammatory prostaglandin, PGE₂, and the gastrotoxic, proinflammatory leukotriene LTB₄ [59]. Licofelone (100 mg/kg) significantly decreased PGE₂ levels to a similar extent as the non-selective NSAIDs, diclofenac and indomethacin (*p* < 0.001). However, increased levels of LTB₄ in the non-selective NSAID groups indicated a shunt of AA metabolism to the 5-LOX pathway. Licofelone prevented the shunt by inhibiting 5-LOX, and prevented an increase in LTB₄ levels [59]. In this model, LTB₄ levels in the licofelone group were equivalent to those in the control group (2.5-0.4 pg/mg protein), but in the diclofenac and indomethacin groups LTB₄ levels were elevated (9.2-2.3 and 8.8-1.6 pg/mg protein, respectively; *p* < 0.001). In addition to the role of LTs and PGs in GI damage/protection, there is evidence to suggest that the adhesion of leucocytes to mesenteric venules could play a role in the development of NSAID-induced GI damage [60,61]. This could be caused by leucocyte chemotaxis due to increased LT levels following a shunt in AA metabolism to the 5-LOX pathway. A study was conducted to compare the adherence of leucocytes to mesenteric venules in rats treated with indomethacin or licofelone [62]. Within 60 min of indomethacin administration, there was a substantial increase in leucocyte adhesion. This increase continued over the course of the study (120 min), and was statistically significant when compared with the control (*p* < 0.05). Conversely, licofelone was associated with only a small increase in adhesion, which did not increase further after 90 min.

Cyclooxygenase Inhibition and Cardiovascular Safety

As discussed, selective COX-2 inhibitors, in addition to having potential GI and renal side-effects, also have potential cardiovascular side-effects. The activity of COX-2 in the endothelium results in the production of cardioprotective PGI₂, which inhibits platelet aggregation, leucocyte activation and adhesion, and accumulation of cholesterol in vascular cells [63]. These effects are antagonized, however, by the activity of COX-1, which is expressed in platelets. COX-1 produces TXA₂, which causes vasoconstriction and platelet aggregation. It is therefore possible that the selective inhibition of COX-2 might lead to an imbalance in the levels of PGI₂ and TXA₂, and possibly to an increase in thromboembolic events [64]. In theory, these thromboembolic events are unlikely to occur with licofelone because inhibition of COX-1 and COX-2, as well as 5-LOX, would theoretically prevent an imbalance between TXA₂ and PGI₂. Furthermore,

licofelone is not known to affect the levels of endocannabinoids, which have vasodilatory properties [65]. Studies are required to further investigate the potential for cardiovascular side-effects with selective COX-2 inhibitors and the cardiovascular safety of licoferone.

CUNCLUSION

Clinical studies have demonstrated that licoferone is effective in the treatment of OA and is comparable to the conventional NSAIDs naproxen and diclofenac. Licoferone is well tolerated and has fewer side effects than naproxen and diclofenac. These results suggest that dual inhibition of COX and 5-LOX may reduce the undesirable GI side effects associated with NSAIDs. Although it has been suggested that the rate of adverse events associated with licoferone may be comparable to selective COX-2 inhibitors with the possibility of less risk of cardiovascular and thromboembolic complications, there is no direct clinical evidence to support this and further trials are needed to test it. In addition, based on pharmacological studies, it has been suggested that licoferone may be a disease-modifying drug; however, this will need to be determined in well-designed trials. If these claims are proven, licoferone will become a credible alternative to conventional NSAIDs and selective COX-2 inhibitors in the treatment of OA and capture a substantial proportion of the considerable market for OA therapies. Like conventional NSAIDs, dual COX and 5-LOX inhibitors may also decrease the production of physiological 'housekeeping' PGs, and thus induce potential side effects. The renal side effects of licoferone that occurred in general pharmacological studies should be a matter of concern in further clinical studies.

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