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REVIEW

NOVEL INHIBITORS OF LEUKOTRIENES SYNTHESIS IN A TREATMENT OF INFLAMMATORY PAIN

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Abstract: A search of new analgesic and anti-inflammatory agents is still ongoing. It is well known that an activation of the leukotriene (LT) synthesis is involved in pain signaling. LTs belong to a class of potent pro-inflammatory mediators that are biosynthesized from arachidonic acid (AA) inter alia by 5-lipoxygenase (5-LOX) enzyme in association with 5-LOX-activating protein (FLAP). Therefore, the main goal of this article review was to present recent advances on new compounds influencing the LOX pathway, which are in clinical studies. The mechanisms of action and possible implementations of these molecules in a treatment of inflammatory pain are discussed.

Key words: leukotriene, lipoxygenase inhibitor, 5-lipoxygenase-activating inhibitor, novel agent

A treatment of chronic pain is still a problem of medicine. As the effectiveness of available analgesics often seems not sufficient, nowadays, there is a search for compounds focused on new mechanisms of action. It was shown that an inhibition of lipoxygenase pathway decrease alleviates not only inflammatory (1-4) or chronic (5, 6) but also acute pain (1, 7). Moreover, it was revealed that increased expression of leukotriene (LT) synthases [5-lipoxygenase (5-LOX), 5-lipoxygenase-activating protein (FLAP), LTA4 hydrolase – LTA4h and LTC4 synthase – LTC4s] and leukotriene B4 type 1 receptors (BLT1) as well as cysteinyl LT type 1 receptors (CysLT1) occurs in spinal cord after peripheral nerve injury (8). Therefore, lipoxygenase inhibitors seem to be promising target of pain therapy.

Leukotrienes (LTs) are considered to play a significant role among others in the pathomechanism of chronic inflammatory disorders, cardiovascular and neurodegenerative diseases and certain types of cancer (9-11). The oxidation of arachidonic acid (AA) by 5-LOX in the presence of 5-lipoxygenase-activating protein (FLAP), which increases the affinity of 5-LOX to AA, is one of the substantial pathways of LTs production (12). 5-LOX converts AA to leukotriene A4 (LTA4), which is further enzymatically transformed into leukotrienes C4 (LTC4), D4 (LTD4) and E4 (LTE4). This group of LTs is called cysteinyl leukotrienes, in contrast to leukotriene B4 (LTB4) which is formed from LTA4 by LTA4 hydrolase (13) (Fig. 1). There are three types of cysteinyl leukotrienes receptors: CysLT1 (located in leukocytes, airway smooth muscles, spleen), CysLT2 (heart, brain, central nervous system, placenta, spleen, leukocytes) (14) and GPR17 (brain, heart and kidney) (15), and three types of receptors for LTB4: BLT1 (located on leukocytes), BLT2 (leukocytes, spleen, liver, ovary) and peroxisome proliferator-activated receptors (PPARs) (expressed in nucleus) (16-18). The diversity of LT receptors occurrence may indicate a role of leukotrienes in numerous physiological and pathological conditions. CysLT1 receptors bind with high affinity to LTD4 and less affinity to LTC4 or LTE4. These receptors are involved in bronchoconstriction, mucus secretion and edema in the airways. Therefore, selective CysLT1 antagonists, such as zafirlukast, montelukast and pranlukast, block the proasthmatic action of the CysLT1. On the other hand, CysLT2 receptors contribute to inflammation, vascular permeability as well as tissue fibrosis. Specific antagonists of CysLT2 receptors have been not known so far, but these receptors bind with equal affinity to LTC4 and LTD4 and with less affinity to

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LTE4. Leukotrienes B4 bind with BLT1, BLT2 and PPARs receptors, however with the higher affinity to BLT1 receptor (19). BLT1 receptors mediate of its chemoattractant and proinflammatory action (12, 14). Little is known about BLT2 physiological function, but recent studies had shown a protective role of the BLT2 receptor in intestinal and airway inflammation (20). Moreover, PPARs function as lipid homeostasis factors and controls the inflammatory responses (18).

Efforts of creating new drugs inhibiting LOX pathway are focused on three targets: inhibition of enzyme, blocking of leukotrienes receptors or inhibition of FLAP. This review summarizes the current state of knowledge on the new LTs inhibitors and possibility of their implementation in pain treatment.

Non-selective inhibitor of COX1/2 and 5-LOX
UP446

UP446 is a standardized blend of extracts from two botanical sources (*Scutellaria baicalensis* and *Acacia catechu*) that contains free B-ring flavonoids and flavans standardized to baicalin and catechin. In

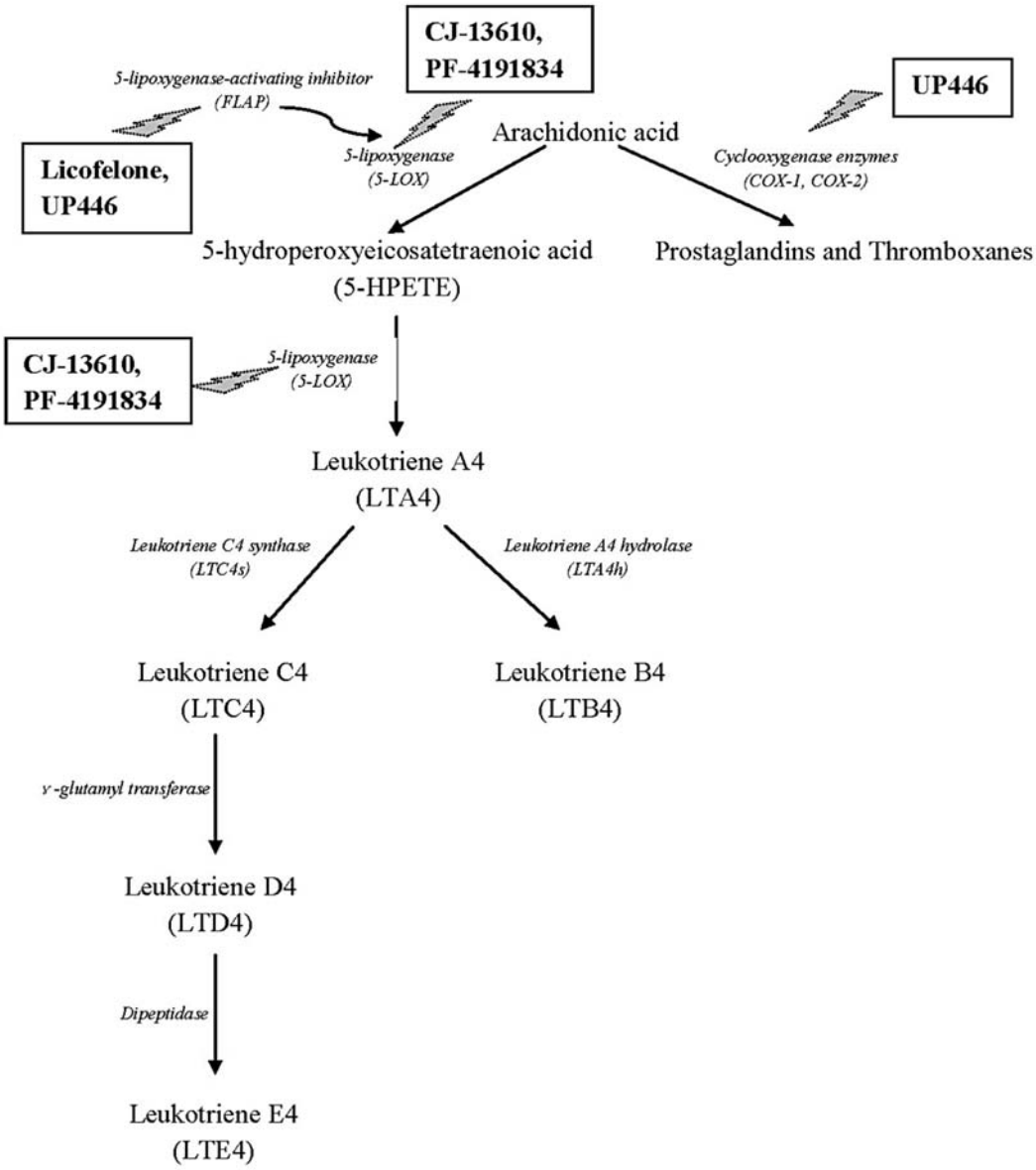

Figure 1. Casdcade of leukotrienes production from arachidonic acid with combination of mechanism of action the new agents investigated in clinical trials

preclinical studies it was shown to induce dual inhibition of COX1/2 and 5-LOX enzymes and to decrease pain in several animal models of inflammation (21, 22). Its effectiveness and safety has been also investigated in a long-term, randomized, double blind, placebo pilot study in 60 osteoarthritis (OA) volunteers and compared to celecoxib, a selective COX-2 inhibitor. The patient's physical condition and visual signs, as well as chemistry laboratory studies, hematology, thrombin time, fecal occult blood, treatment adverse events were monitored for safety. Its effectiveness was estimated with two questionnaires: The Western Ontario and McMaster Universities Arthritis Index (WOMAC) and the 36-Item Short Form Health Survey from the Medical Outcomes Study (MOS-SF 36) at 0, 30, 60 and 90 days of trial and compared to celecoxib or placebo. This study demonstrated that UP446 (250 mg/day and 500 mg/day) appeared to be more effective than celecoxib at a dose of 200 mg/day in the reduction of function incapacity caused by osteoarthritis within 30 days of treatment and UP446 at the higher dose was more effective than celecoxib in reduction of pain at 30 day. Laboratory measures of anti-inflammatory efficacy, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were not clinically significant for any group. Fecal occult blood was reported comparably in each treatment group (22). Recent clinical trial has also confirmed the effectiveness of the UP446 (500 mg/day) in decreasing knee joint pain and stiffness in comparison to naproxen (440 mg/day), a non-selective COX-1 and COX-2 inhibitor, in patients with mild to moderate osteoarthritis as early as after 1 week treatment (23). These trials show that dual COX/LOX inhibition in OA treatment may provide clinical safety and efficacy benefits. However, the additional study in larger patient population is necessary to assess the further safety and efficacy of UP446.

Selective 5-LOX inhibitors

CJ-13610

CJ-13610 with high selectivity blocks 5-LOX enzyme through a non-redox, non-iron chelating mechanism as a direct competitor of arachidonic acid (24). In preclinical studies, the compound showed antinociceptive and anti-inflammatory effects in the acute model of carrageenan-induced paw edema and in the chronic model using complete Freund's adjuvant. Furthermore, the compound was tested in osteoarthritis pain model using a medial meniscal transaction. In this study, CJ-13610 showed efficacy in relieving pain after acute administration as well as after 4 days of treatment (25). CJ-13610 was investigated in order to estimate the pharmacokinetic properties in the I phase of clinical trials. Matthew (26) reported that the drug molecule was metabolized by CYP3A cytochrome, the predominant metabolic pathway was oxidation of the sulfur heteroatom and the pharmacokinetic profile of the CJ-13610 was predicted after single-dose administration in humans. Sutton (27) performed the pharmacokinetic studies of a controlled release form of CJ-13610. In order to improve the pharmaceutical properties of CJ-13610 the derivative compound, selective and non-redox type 5-LOX inhibitor – PF-4191834 was designed.

PF-4191834

PF-4191834 is a competitive inhibitor of the 5-LOX enzyme that is being developed as an oral anti-inflammatory therapy for the treatment of asthma. It was shown that PF-4191834 has strong inhibiting properties, with about 300-fold selectivity for 5-LOX over 12-LOX and 15-LOX and no influence on the COX enzymes in *in vitro* studies. Further *in vivo* study confirmed inhibition of leukotrienes (CysLT and LTB4) in the carrageenan-inflamed air-pouch model in rats in a dose-dependent manner after single and 7 days administration at a dose of 5 mg/kg. PF-4191834 also demonstrated strong efficacy in chronic inflammatory pain. This effect was similar to the effect observed by celecoxib, a selective COX-2 inhibitor (28). In spite of the fact that pre-clinical results were promising, the II phase of clinical trials was terminated in osteoarthritis patients due to serious adverse events (SAEs): gastric ulcer hemorrhage and acute hepatitis (29). In this study SAEs occurred in the group receiving combination of PF-4191834 with naproxen. Therefore, it remains unclear if those adverse effects were related to PF-4191834 alone or to combined administration with naproxen. However, PF-4191834 is being investigated in clinical trial in asthma patients and it successfully completed the II phase study without SAEs (29).

5-Lipooxygenase activating protein inhibitors (FLAP inhibitors)

Licofelone (ML3000)

Originally discovered by Merckle GmbH and developed by EuroAlliance for osteoarthritis treatment as a dual COX/5-LOX inhibitor, recently it was characterized as a FLAP inhibitor (29) and weak inhibitor of microsomal prostaglandin E synthase-1 (mPGES-1) (30). It has been widely examined in animal models of inflammation and pain, as

well as it passed III phase of clinical trials for osteoarthritis treatment (31). In preclinical studies, it completely blocked secretion of PGE_2 and LTB4 in carageenan-induced rat paw edema model (32). Furthermore, licofelone was as effective as indomethacin in reducing hyperalgesia in carrageenan-, bradykinin-, and arachidonic acid-induced rat hind paw edema models (33). Compound demonstrated longer action and stronger antinociceptive effect in comparison to indomethacin and zileuton in rat model of incisional pain (34). It also showed neuroprotective (35), antipyretic (34), antithrombotic (36) and antibronchoconstrictive effect (37).

Clinical studies were performed in healthy adults in order to assess the tolerability of ML3000 and in OA patients in order to assess the safety and efficacy. In phase I of clinical trial licofelone was assessed at two doses of 200 and 400 mg twice a day (bis in die – *bid*) for 4 weeks in comparison to naproxen at a dose of 500 mg *bid* or placebo in 121 healthy volunteers. In the study it was found that gastric mucosa was normal in 93% and 89% of volunteers given licofelone at doses of 200 and 400 mg *bid*, respectively, in 90% given placebo and 37% given naproxen at a dose of 500 mg *bid* (38). Therefore, gastric toxicity of licofelone appeared to be at the similar level to placebo and less than of naproxen. Pharmacokinetic parameters were explored in other study (39). Licofelone was administrated to 18 healthy young males and females (the average age was 30.9) and elderly (the average age was 72.1) individuals twice a day at a dose of 200 mg for 5 days and at a single dose of 200 mg at day 6 of experiment. It was found that the maximum plasma concentration (C max) was 0.74 in 4 h after administration, the rate of systemic elimination – 11.1 ± 7.0 in young individuals and 8.7 ± 4.7 in elderly individuals, as well as the mean $t_{1/2}$ value was 15% higher in the elderly study population.

Subsequent phase II of clinical studies evaluated the efficacy of this compound at various dosages in OA patients. In the first study, patients n = 107 received licofelone at doses of 100, 200 and 400 mg *bid* or placebo for 4 weeks (38). This study found that tested compound at doses of 200 or 400 mg *bid* was effective in relieving pain and stiffness as determined by WOMAC index, as well as it showed effectiveness in secondary endpoints, such as level of disability. In the second trial, 404 patients were treated with licofelone at the same doses as in the previous study and the effect of the drug was compared to placebo or diclofenac (50 mg, three times a day) (38). Licofelone resulted in superior analgetic effect and greater improvements in stiffness as well as disability in comparison with placebo, but no significant difference was noted between diclofenac and licofelone. The most frequent side effects were diarrhea and abdominal pain at a dose of 400 mg.

The safety and efficacy of licofelone at a dose of 200 mg *bid* or naproxen at a dose of 500 mg *bid* for 12 weeks was tested in phase III of study. In 148 OA subjects efficacy was similar in both groups (40). However, licofelone has proven to have better gastrointestinal tolerability (14% of licofelone-treated patients reported gastrointestinal side effects *vs* 26% of those treated by naproxen). In a 52-week, long term phase III study with a large cohort of patients (n = 710) the efficacy and safety of licofelone (100 or 200 mg *bid*) was compared to naproxen (500 mg *bid*) in knee OA patients (38). The results of the study revealed that licofelone had an improved efficacy throughout the 52 weeks, better gastrointestinal profile and less risk of hypertension aggravation comparing to naproxen.

This data suggest that licofelone may be a promising alternative to traditional non-steroidal anti-inflammatory drugs not only in osteoarthritis treatment, but also in co-treatment with other drugs from different pharmacological groups in reducing inflammatory and neuropathic pain. Double COX/LOX pathway inhibitors possess synergistic analgesic effect due to blocking of arachidonic acid metabolism and subsequent inflammatory process. Therefore, they inhibition of prostaglandins (products of COX) as well as leukotrienes (products of 5-LOX) synthesis may result in stronger anti-inflammatory effect. Moreover, double inhibiting strategy seems to be reasonable due to better gastrointestinal and cardiovascular tolerability, as well as significantly diminish the possibility of drug-drug interaction.

CONCLUSIONS

LOX pathways seem to be involved in pathogenesis of inflammatory pain. Moreover, data available in the literature indicate that not only 5-LOX inhibitors, but also FLAP inhibitors, may be promising in a treatment of this kind of pain. Noteworthy, they are also dual COX/LOX inhibitors, as they possess not only a more potent analgesic activity but also less side effects in comparison to selective and not selective COX-1/COX-2 inhibitors.

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