INHIBITORS OF LEUKOTRIENES SYNTHESIS: NOVEL AGENTS AND THEIR IMPLEMENTATION

KRYSYTNA CEGIELSKA-PERUN, EWA MARCZUK and MAGDALENA BUJALSKA-ZADROŻNY*

Department of Pharmacodynamics, Centre for Preclinical Research and Technology (CePT) Laboratory, Medical University of Warsaw, , Banacha 1b St., 02-097 Warszawa, Poland

Abstract: Leukotrienes (LTs) belong to 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). An activation of LTs synthesis pathway occurs during the development and maintenance of numerous diseases such as asthma, anaphylactic shock, allergic rhinitis, psoriasis, rheumatoid arthritis, osteoporosis, as well as cardiovascular diseases, neurodegenerative diseases and certain types of cancer. The main goal of this review was to present recent advances on the new compounds influencing the LOX pathway, which are undergoing clinical studies. The mechanisms of action and possible implementations of these molecules in a treatment of asthma, cancer and cardiovascular diseases are discussed.

Keywords: leukotriene, lipoxygenase inhibitor, 5-lipoxygenase-activating inhibitor, novel agent

Leukotrienes (LTs) are considered to play a significant role in the pathomechanism in plenty of diseases, such as bronchial asthma, allergic rhinitis, psoriasis, rheumatoid arthritis, osteoporosis, as well as cardiovascular diseases, neurodegenerative diseases and certain types of cancer (1-3). It is commonly known that one of the substantial pathways of LTs production is associated with the oxidation of arachidonic acid (AA) by 5-lipoxygenase (LOX) using 5-lipoxygenase-activating protein (FLAP) which increases the affinity of 5-LOX to AA (4). 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 (5). There are two types of cysteinyi leukotrienes receptors: CysLT1 (located in leukocytes, airway smooth muscles, spleen), and CysLT2 (heart, brain, central nervous system, placenta, spleen, leukocytes) and two types of receptors for LTB4: BLT1 (located on leukocytes) and BLT2 (leukocytes, spleen, liver, ovary) (6, 7). The latest studies suggest the existence of the third type of cysteinyi LT receptor (8). 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 not been known so far, but these receptors bind with equal affinity to LTC4 and LTD4 and with less affinity to LTE4. BLT1 receptors mediate of its chemoattractant and proinflammatory action. However, little is known about BLT2 physiological function (4, 8).

Efforts of creating new drugs inhibiting LOX pathway are focused on three targets: inhibition of enzyme, blocking of leukotrienes receptors or inhibition of FLAP, a protein involved into activation and/or presentation of AA to the enzyme. This review summarizes the current knowledge on the new LTs inhibitors and possibility of their implementation in the selected diseases (Fig. 1).

Novel inhibitors of leukotrienes synthesis in asthma treatment

In asthma process a notable increase in inflammatory mediators and multiple cytokines is observed,
as well as the up-regulation of 5-LOX activity and FLAP expression is well-documented (9). Furthermore, the latest studies have shown that an increasing production of the CysLTs in asthma contributes significantly to exacerbations of asthma symptoms (8). Nowadays, two drugs blocking LOX pathway in asthma treatment: zileuton, a 5-LOX inhibitor, and a group of CysLT1 receptor blockers named lukasts (zafrirlukast, montelukast, pranlukast etc.) are available. Unfortunately, these medicaments possess numerous drawbacks. Montelukast has limited mechanism of action as it blocks only CysLT1 receptor. In turn, zileuton possesses adverse pharmacodynamics effects, it may produce aminotransferase elevations and numerous interactions with other drugs, inter alia, theophylline. Moreover, zileuton immediate-release form was withdrawn in 2008, while the modified-release form is still available (outside Poland). Additionally, it is difficult to predict responsiveness to antileukotriene therapy in the indi-

Figure 1. Chemical structures of leukotrienes synthesis inhibitors
Furthermore, a product of 5-LOX activity, 5-hydroxyeicosatetraenoic acid (5-HETE), plays also a role in angiogenesis by an induction of vascular endothelial growth factor (VEGF) expression in colon cancer. VEGF is considered to be the most potent tumor angiogenic factor. Some reports suggest that elevated 5-LOX expression results in disturbance in metalloproteinase activity, which leads to extracellular matrix destruction and enhances metastasis. This phenomenon reduces survival in animal models of cancer and has shown to increase the invasiveness of many cancer cell types in human, for example, head and neck cancer. Moreover, suppression of 5-LOX in some studies increased chemosensitivity of cancer cells, which means that it may condition tumor response to chemotherapeutic agents. All those factors together contribute to an important role of LOX in human prostate, pancreatic, colon, bladder, testicular, esophageal, renal, hepato-ma, lung and breast cancers. While the pro-carcinogenic result of 5-LOX overexpression is well documented, the role of 15-LOX seems to be controversial. In some animal models and in human colorectal and prostate cancer cells, 15-LOX was found to suppress tumor growth, however, data still remain discrepant. Therefore, in the article we present recent developments in anti-LOX strategy in the cancer therapy. Furthermore, dual COX/LOX inhibitors are in particular interest of scientist, since it is known that they act synergistically in inflammation-related cancer prevention and treatment.

**Zileuton + imatinib**

Zileuton was introduced in USA in 1996 by Abbott Laboratories for asthma treatment and is now marketed by Cornerstone Therapeutics Inc. under the brand name ZYFLO. Recently, efficacy of zileuton in other diseases than asthma is being investigated. It is in the II phase of clinical trials for acne vulgaris treatment conducted by Clinical Therapeutics (25). Emerging evidence suggests that it may be effective in chronic myelogenous leukemia (CML) in co-treatment with imatinib, a tyrosine-kinase inhibitor. Imatinib has been used in treatment of multiple cancers, especially Philadelphia chromosome-positive (Ph+) CML (26). Although imatinib proved to be an excellent treatment option for patients with CML, it was found that about one-third of patients remain resistant or intolerant (27). In animal studies, it has been revealed that in knockout mice without Alox5 (5-lipoxygenase coding gene) in leukemia-stem cells CML did not develop, which suggests 5-LOX significance in leukemia genesis. Therefore, treatment of CML mice with zileuton reduced CML stem cells and pro-
longed the survival of CML mice (28). Furthermore, combined use of zileuton with imatinib appeared to be more effective than each drug in monotherapy. Zileuton and imatinib combination in CML treatment is currently in a phase I of clinical study conducted by University of Massachusetts, Worcester.

**Auranofin**

Auranofin (AUR) is a gold complex used in rheumatoid arthritis treatment. However, the exact mechanism of action of gold compounds still remains unknown; it is believed that they act through modulation of autoimmune system (29, 30). In an *in vitro* study it was shown that AUR acted as a 5-LOX inhibitor in human polymorphonuclear cells (PMNs). It also reduced chemotaxis of PMNs towards LTB4 (31). This activity may contribute to efficacy of auranofin in OA treatment. Recently, its activity and efficacy is being examined in few clinical trials: in chronic lymphocytic leukemia patients (CLL) and in patients with recurrent epithelial ovarian, primary peritoneal or fallopian tube cancer. Another studies evaluated the effectiveness of AUR in HIV patients, in paclitaxel-induced pain syndrome and in squamous cell lung cancer (in co-administration with sirolimus, an immunosuppressive drug). These studies are in recruitment phase, while the clinical trial in which the combined administration of AUR with sirolimus in patients with advanced solid tumors was planned (data obtained from www.clinicaltrials.gov).

**NDGA**

In the study of Tong et al. (32) the efficacy of NDGA (nordihydroguaiaretic acid), a 5-LOX inhibitor, was tested in two breast cancer cell lines: MCF-7 (estrogen receptor positive, ER+) and MDA-MB-231 (estrogen receptor negative, ER-). The influence on breast cancer cells proliferation as well as mechanism of action were investigated. The study revealed that NDGA inhibited growth and induced apoptosis in both cell lines, suggesting that its activity is independent from estrogen receptor presence. Inhibition of LOX pathway led to induction of intrinsic apoptosis through cytochrome C release from mitochondrial membrane and subsequent activation of caspase cascade. Moreover, NDGA decreased the level of Bcl-2 and Mcl-1 proteins, known as the anti-apoptotic factors, and increased the level of pro-apoptotic bax protein in both cell lines. It is known that carcinogenesis depends on pro- and anti-apoptotic proteins balance (33). Therefore, NDGA leads to cancer cells death by acting on LOX, Bcl-2, Mcl-1 and bax proteins. Nowadays, NDGA has been examined in clinical trials in other cancer types. In II phase study conducted by University of California, San Francisco, NDGA in oral daily dose 2000 mg did not decline the specific antigen (PSA) level in 12 non-metastatic prostate cancer patients. The study has been terminated after 12 weeks of the observation due to lack of the desired effect. In other I/II phase clinical study conducted by Sidney Kimmel Comprehensive Cancer Center the activity of tetra-O-methyl nordihydroguaiaretic acid (terameprocol/EM-1421), an inhibitor of Sp-1 mediated surviving transcription, after intravenous (*i.v.*) administration has been examined in 35 patients with recurrent high-grade glioma. However, the results of the study have not been published. EM-1421 has also been investigated in recurrent or refractory solid tumors, in cervical intraepithelial neoplasia, in malignant tumors of head and neck and in leukemia treatment by Erimos Pharmaceuticals (data obtained from www.clinicaltrials.gov).

**Curcumin**

Curcumin is a natural phenol compound derived from *Curcuma longa* (Zingiberaceae) rhizoma powder, which is also known as turmeric. It has been used in traditional Asian cuisine as a spice and medicine for thousands of years. Due to its anti-inflammatory, analgetic and anti-microbial activity it has been used in traditional Chinese and Indian medicine in treatment of disorders such as anorexia, biliary and hepatic disorders, cough and sore throat, diabetic wounds, rheumatism and sinusitis (34). Over the last decades, curcumin properties were extensively investigated in the *in vitro* and *in vivo* animal models, as well as in clinical trials. It has been proven that it reduces blood cholesterol, prevents LDL oxidation, prevents aggregation of platelets, thrombosis and myocardial infarction, reduces symptoms of type II diabetes, rheumatoid arthritis, Alzheimer’s disease and inhibits HIV replication (35). Moreover, its anti-cancer activity seems to be well documented in different types of cancer models.

Such a wide range of curcumin therapeutic properties results from diversified biochemical activity. It is known that curcumin blocks arachidonic acid metabolism by COX-2 and 5-LOX inhibition (36, 37), and as a result prevents subsequent inflammatory state development. Moreover, in numerous *in vitro* models of cancer it proved to have antiproliferative, apoptotic, antimetastatic and antiangiogenic properties. Antitumor activity results from regulation of many transcription factors (for instance, NF-κB,
Based on the results of the studies, 5-LOX inhibitors may represent a promising group of drugs in complementing cancer polytherapy. Furthermore, they seem to be a good strategy in cancer prevention in the high-risk patients.

**Leukotriene modifiers in cardiovascular diseases treatment**

Cardiovascular disease (CD) is one of the main causes of death worldwide. Epidemiological data show that in USA heart disease (620 000 of 2.4 million total deaths) and stroke (135 000 deaths) are the first and third leading causes of death (39). In spite of the fact that medical progress of last 50 years in CD prevention, diagnostic methods and pharmacological treatment led to decrease of mortality rate, it still remains a burning issue.

Invention of statins is certainly a milestone in cardiology as it prevents from atherosclerosis and its complications. Atherogenesis is a chronic process which includes arterial wall injury, lipid accumulation and oxidation, aberrant immune and inflammatory reactions. A role of inflammation and lipoxigenase pathway in atherogenesis has been investigated in numerous studies. It is well established that LT mediators play a significant role in vascular inflammation. LTB4 is a strong chemoattractant of neutrophils and T cells, it promotes adhesion of leucocytes to vascular endothelium, increases vascular permeability and promotes smooth muscle cells proliferation and migration (40, 41). CysLTs are micro vessels constrictors, they enhance permeability, reduce myocardial contractility and blood flow (42), they also play role in ischemia and shock (43). The influence of leukotriene pathway on atherogenesis was proven in arterial walls of patients with atherosclerosis (44). Clinical trial on asthmatic patients taking montelucast, a CysLT receptor antagonist, showed that LT receptor blocking results in decrease of c-reactive protein (CRP), total cholesterol, LDL, HDL and triglycerides (45). Moreover, there are some reports from epidemiological studies on FLAP single nucleotide polymorphism (SNP) that indicate the role of different FLAP haplotypes in cardiovascular risk in Chinese (46) and Icelanders (47), but another study on large European population finds no association (48). The exact mechanism of leukotriene action in atherogenesis and other cardiovascular diseases seems to leave many to discover, nevertheless yet it became a subject of interest in cardiologic drug research.

**Atreleuton (Via-2291)**

Atreleuton is a 5-LOX inhibitor introduced by VIA Pharmaceuticals and has completed the II
phase of clinical trials in vascular inflammation in patients after acute coronary syndrome event. In randomized, placebo trial in 56 post-acute coronary syndrome patients, after 6 months of administration, it significantly decreased the LTE4, high-sensitivity CRP (hs-CRP) levels, coronary plaque volume (PV) and increased left ventricular ejection fraction (LVEF). The study revealed that the decrease in necrotic core PV and increase of LVEF were correlated with decrease of LTE4 level (49).

**Veliflapon (DG-031)**

Introduced by deCode genetics, veliflapon, a FLAP inhibitor, has successfully Phase I and Phase II of clinical trials in myocardial infarction and stroke patients. The results of these studies demonstrated that veliflapon was well-tolerated and considerably reduced production of LTB4 level in a dose-dependent manner. The Phase III study has been conducted on African-American patients with acute coronary syndrome, as this population has the highest risk for myocardial infarction developing due to unfavorable FLAP and leukotriene A4 hydrolase (LTA4H) genes haplotypes. Unfortunately, in 2006 deCode genetics suspended trial due to unexpected tablet formulation problem since too slow dissolution of tablets could adversely influence drug blood concentration and therapeutic effect.

In randomized, placebo, cross-over trial in 191 patients carrying at-risk genes of FLAP/LTA4H treated with DG-031 for 4 weeks (at the doses 250, 500 or 750 mg/day) the 750 mg dose resulted in decrease of biomarkers associated with myocardial infarction (26% LTB4 reduction and 12% myeloperoxidase reduction). Furthermore, two doses 500 and 750 mg also resulted in long-term (in 4th week of wash-out) decrease of CRP (50).

**CONCLUSIONS**

Inflammation is known to be involved in pathogenesis and maintenance of numerous diseases, such as pain, osteoarthritis, bronchial asthma, psoriasis, ulcerative colitis, atherosclerosis, cancer, neurodegenerative diseases and ischemic reperfusion injury. A recent approach to many disease treatment includes multi-target pharmacotherapy and blocking of leukotriene pathway, which seems to be a promising one. In spite of the fact that leukotriene biochemistry leaves much to discover, two medications - zileuton and a group of lukasts - are already in use in asthma treatment, and others are about to finish clinical trials. Not only 5-LOX inhibitors, but also many of FLAP inhibitors, for instance veliflapon, are being in different stages of development, suggesting that FLAP may be a beneficial target for new pharmaceuticals. Future will show how many of today’s ideas for leukotriene antagonists find a practical use.

**REFERENCES**


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