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Mini Review

Drug Discovery from the Arachidonic Acid Cascade: Orally Active Leukotriene D_4 (LTD_4) Antagonists

NAKAI Hisao[1*] (hisaotakako@yahoo.co.jp)
[1] Imex Japan Co., Ltd. Kyoto, Japan.

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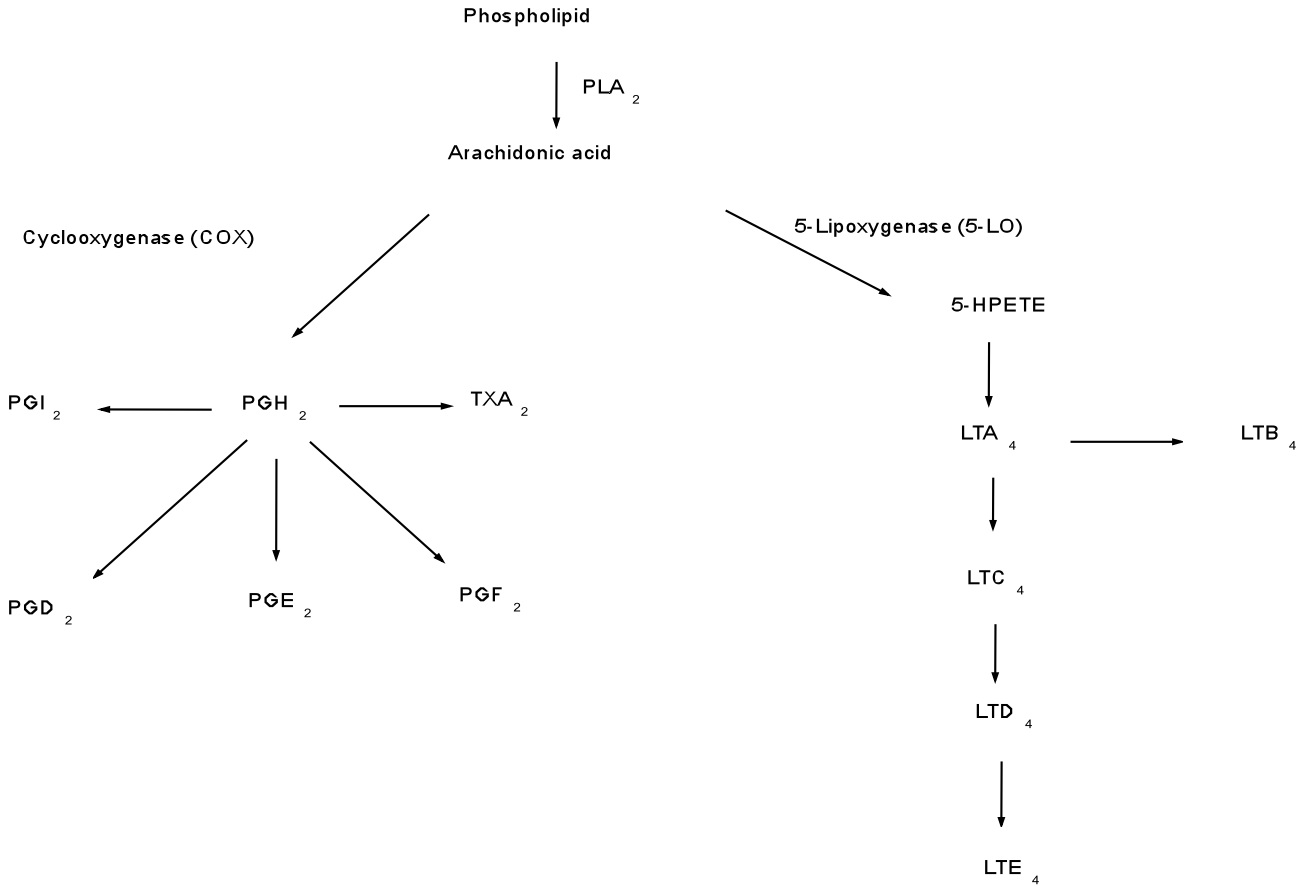
Arachidonic acid cascade

Arachidonic acid (AA) plays a key role as a precursor of various biologically active products, such as prostaglandins (PGs) and leukotrienes (LTs). Prostanoids (PGs and LTs) are derived from AA *via* the intermediacy of cyclooxygenase and lipoxygenase products, respectively (Scheme 1).

The metabolic pathway of AA, consisting of prostanoid biosynthesis (Scheme 1), is called the "AA cascade" because it looks like "a cascade" which flows down from AAs to the metabolites. Since the AA cascade was elucidated, enzymes for biosynthesis and LT receptors: a) phospholipase A_2 (PLA_2); b) cyclooxygenases I (COX-I) and II (COX-II); c) PG receptors; d) thromboxane A_2 (TXA_2) receptors and synthase; e) 5-lipoxygenase; and f) LT receptor have been the intriguing drug discovery targets. Many drugs have been developed from the AA cascade, including LT receptor antagonists.

i) COX route: Prostanoids (PGs and TXA_2) are oxidative metabolites of AA, which is a C-20 polyunsaturated fatty acid released from phospholipids by PLA_2. Oxidative metabolism of AA by COX results in endoperoxide (PGH_2 *via* PGG_2), which is further transformed to PGs and TXA_2. The pathways of PGs and TXA_2 biosynthesis, starting from AA, are shown in Scheme 2. It is well known that PGs and TXA_2 play important roles in maintaining homeostasis in the living body. For example, VIOXX and Celebrex (COX-II inhibitors), and CATACLOT (a TXA_2 synthase inhibitor) have been discovered to be clinically useful. Besides, conventional analgesics such as aspirin, indomethacin, ibuprofen and others are, in fact, non-selective inhibitors of COX-I and COX-II.

Efficient chemical synthesis of PGs[1,2] was a crucial concern because production of sufficient supplies was exclusively dependent on their chemical synthesis, as they are difficult to be procured from natural sources.

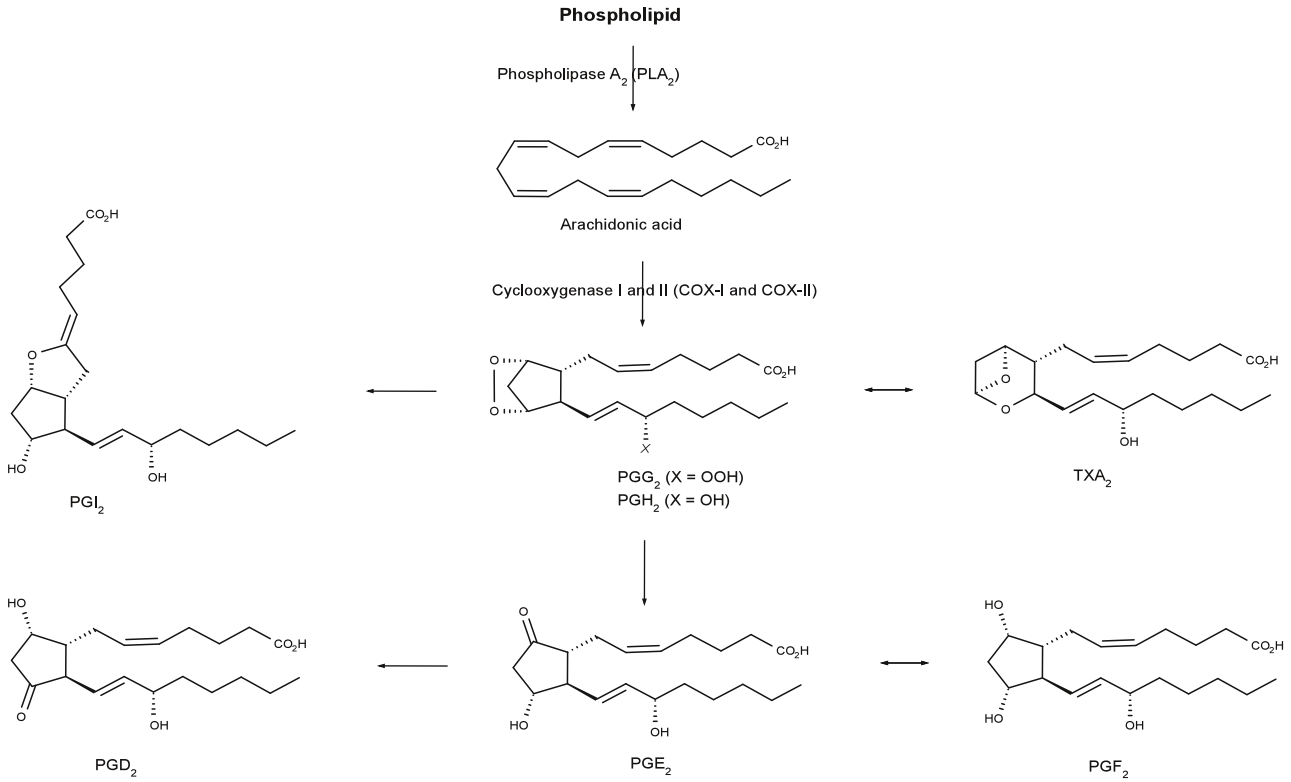


Scheme 1: Biosynthesis of prostanoids (PGs & TXs) and leukotrienes (LTs)

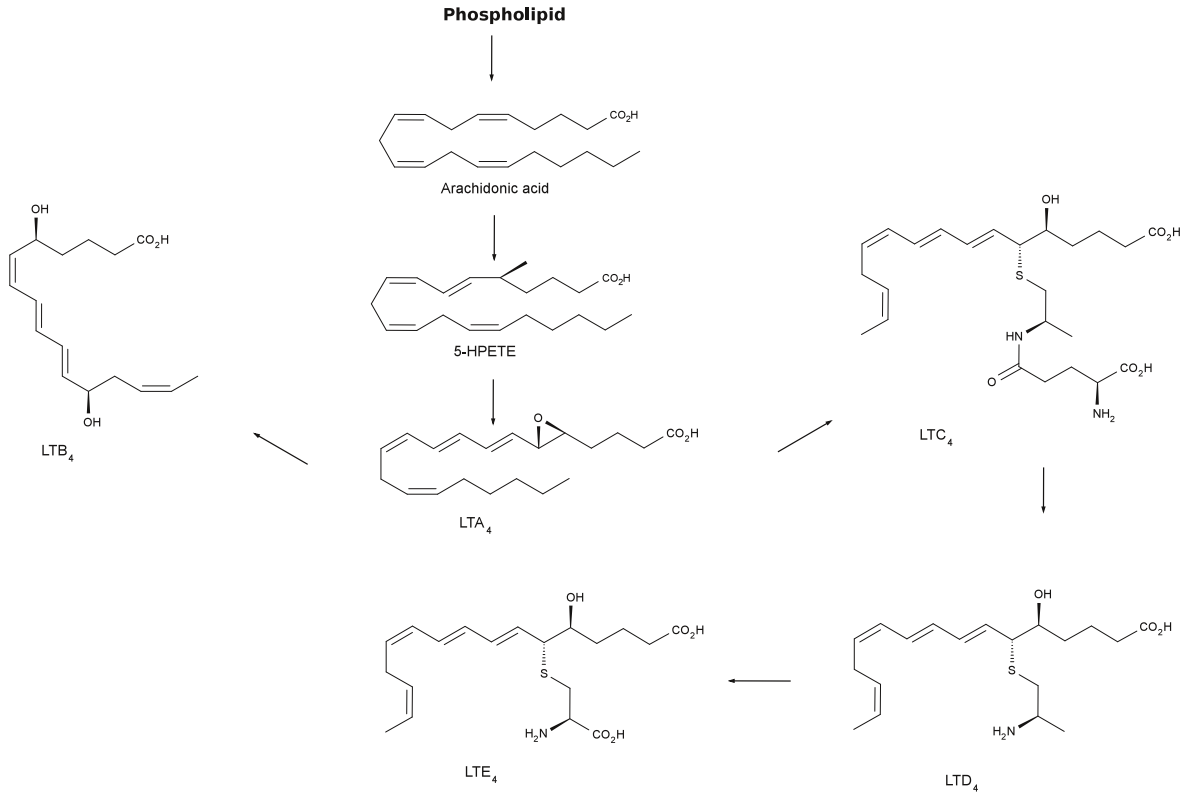
Efficient chemical synthesis of PGs in their optically active forms resulted in the elucidation of their biological activity followed by the discovery of related novel clinically useful therapeutic drugs (e.g. PG agonists and their biosynthesis inhibitors). Limaprost, Beraprost, Latanoprost and Tafluprost (PG agonists) are the typical examples.

With regard to the chemical synthesis of PGs, two typical and important synthetic pathways were first reported by Corey et al. (1969) and Noyori et al. (1982).

ii) 5-Lipoxygenase route (LTs): LTC_4, LTD_4, and LTE_4 - identified as components of slow reacting substances of anaphylaxis (SRS-A) - have been shown to cause potent



Scheme 2: Biosynthesis of Prostanoids (PGs)



Scheme 3: Biosynthesis of leukotrienes (LTs)

bronchoconstriction, increase microvascular permeability, and alter mucous production and transport. LTB_4 stimulates neutrophil aggregation, as well as chemotaxis[4] and chemokinesis, and has been shown to be an endogenous mediator of various *in vivo* inflammatory responses.

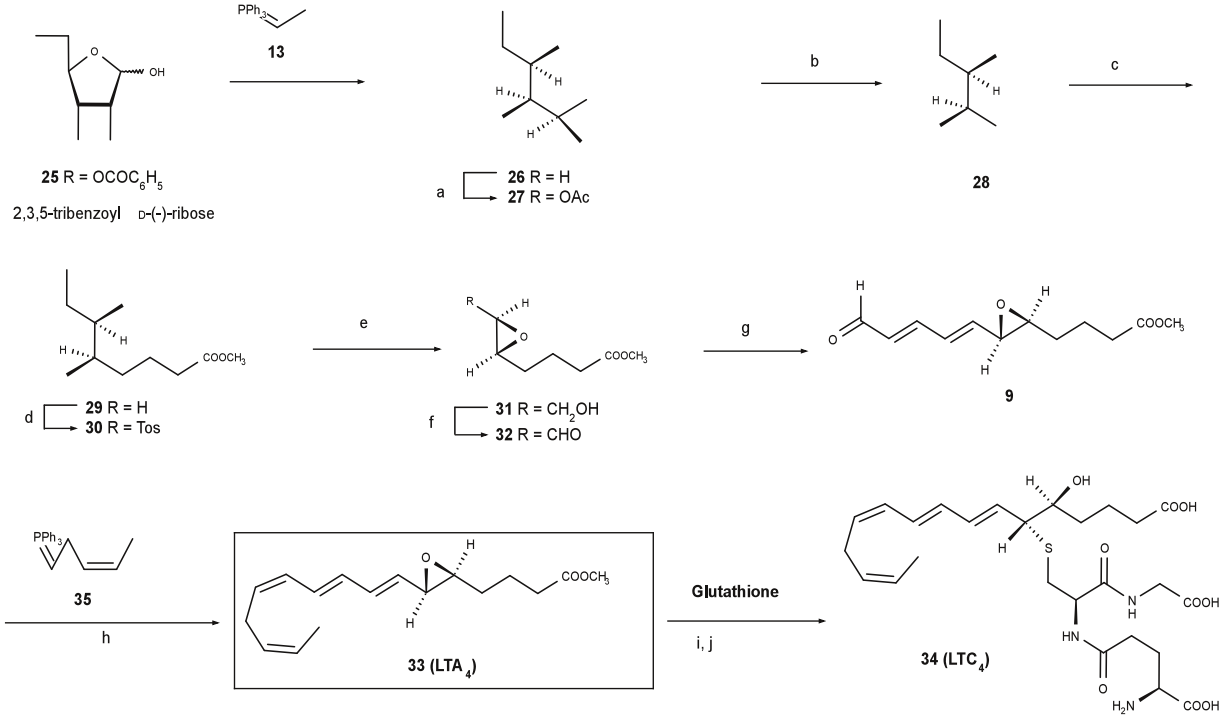
Chemical synthesis of LTs:[3),4)] In a similar perspective to PGs, the efficient chemical synthesis of LTs was a crucial concern, because production in sufficient quantities was exclusively dependent on their chemical synthesis, as they too are difficult to obtain from natural sources. Efficient chemical synthesis of LTs in their optically active forms resulted in the elucidation of their biological activity, followed by the discovery of related new clinically useful therapeutic drugs such as LT antagonists and LT biosynthesis inhibitors. The first chemical synthesis of LTs was realized by Corey et al. in 1980 (Scheme 4).

This synthetic process of LTC_4, starting from the 2,3,5-tribenzoyl derivative of D-(-)-ribose, is schematically described in Scheme 4. A key intermediate in both the chemical synthesis and biosynthesis of LTC_4 is (-)-methyl *trans*-5(*S*), 6(*S*)-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoate (LTA_4 methyl ester) **33** (crude), which had previously been surmised to be a precursor (as the corresponding acid) of new eicosanoids and synthesized in the racemic form. Reaction of LTA_4 methyl ester **33** (with higher purity), which includes the corresponding 11-*trans*-isomer as a minor component derived from the Wittig reaction using **35**, with glutathione and triethylamine in methanol, followed by methyl ester cleavage (0.1 M potassium carbonate in methanol) afforded **34** (LTC_4). Similarly, removal of the undesired 11-*trans*-isomer by reversed-phase HPLC afforded **34** (LTC_4) as well. The epoxide **33** has also allowed the synthesis of LTC_4 analogs such as LTD_4 and LTE_4 having the sulfur of cysteinyl glycine and cysteine, respectively, attached to C-6 rather than to glutathione.

Optical resolution was not necessary here because this stereoselective synthesis started with optically active D-(-)-ribose **25** as starting material. As soon as the chemical synthesis of LTs was published, the race was on to search for LT antagonists, which might be expected to be clinically useful.

Discovery of Orally Active LTD_4 Antagonists

Asthma is a common chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction and bronchospams. Common symptoms include wheezing, coughing, chest tightness, and shortness of breath. Asthma is thought to be caused by a combination of genetic and environmental factors. Its diagnosis is usually based on the pattern of symptoms, response to therapy over time and spirometry. It is clinically classified according to the frequency of symptoms, forced expiratory volume in one second (FEV1), and peak expiratory flow rate. Asthma may also be classified as atopic (extrinsic) or non-atopic (intrinsic).



Reagents: a) acetic anhydride, H_2SO_4 (catalytic quantity); b) Zn-Hg/HCl; c) H_2/Pd-C/HCl/MeOH; d) TsCl/pyridine; e) K_2CO_3/MeOH; f) Collins reagent/CH_2Cl_2; g) 1-lithio-4-ethoxybutadiene/THF and then MsCl/Et_3N/CH_2Cl_2/pH 7.0 phosphate buffer; h) **35**; i) glutathione; j) 0.1 M K_2CO_3

Scheme 4: Chemical synthesis of LTC_4

Medications for asthma are divided into two general classes: **a) quick-relief medications** used to treat acute symptoms; and **b) long-term control medications** used to prevent further exacerbation.

a) Quick–relief medications:

i) Short-acting β-2 adrenoceptor agonists (SABAs), such as salbutamol (*albuterol* USAN) are the first-line treatment for asthma symptoms. They are recommended before exercise in those with exercise-induced symptoms.

ii) Anticholinergic medications, such as ipratropium bromide, provide additional benefit when used in combination with SABAs in those with moderate or severe symptoms. Anticholinergic bronchodilators can also be used if a person cannot tolerate SABAs. If a child requires admission to hospital additional ipratropium does not appear to provide additional benefit over SABAs.

iii) Magnesium sulfate intravenous treatment has been shown to provide a bronchodilating effect when used in addition to other treatments for severe acute asthma attacks.

iv) Older, less selective adrenergic agonists, such as inhaled epinephrine, have a similar efficacy as SABAs. They are, however, not recommended for asthmatic treatment due to concerns of excessive cardiac stimulation.

b) Long–term control medications:

i) Corticosteroids are generally considered the most effective treatment available for long-term control. Inhaled forms such as beclomethasone are usually used except in the case of severe persistent disease, where oral corticosteroids may be needed. It is usually recommended that inhaled formulations be used once or twice daily, depending on the severity of symptoms.

ii) Long-acting β-adrenoceptor agonists (LABAs) such as salmeterol and formoterol can improve asthma control, at least in adults, when given in combination with inhaled corticosteroids. In children this benefit is uncertain. When used without steroids they increase the risk of severe side-effects and even with corticosteroids they may slightly increase said risk.

iii) Leukotriene receptor antagonists (such as ONON, Singulair and Accolate) may be used in addition to inhaled corticosteroids, typically also in conjunction with LABAs. Evidence is insufficient to support use in case of acute exacerbation. In children they appear to be of little benefit when added to inhaled steroids. In those under five years of age, they were the preferred supplemental therapy to inhaled corticosteroids by the British Thoracic Society in 2009.

iv) Arachidonate 5-lipoxygenase (5-LOX) enzyme inhibitors, such as zileuton and St John's wort, slow down or stop the production of asthma-related LTs, which promote inflammation, microvascular permeability, bronchoconstriction and mucus secretion. The use of efficacious 5-LOX inhibitors for treating asthma in both monotherapy and combination therapy with LT receptor antagonists is well practiced.

v) Mast cell stabilizers (such as cromolyn sodium) are another non-preferred alternative to corticosteroids.

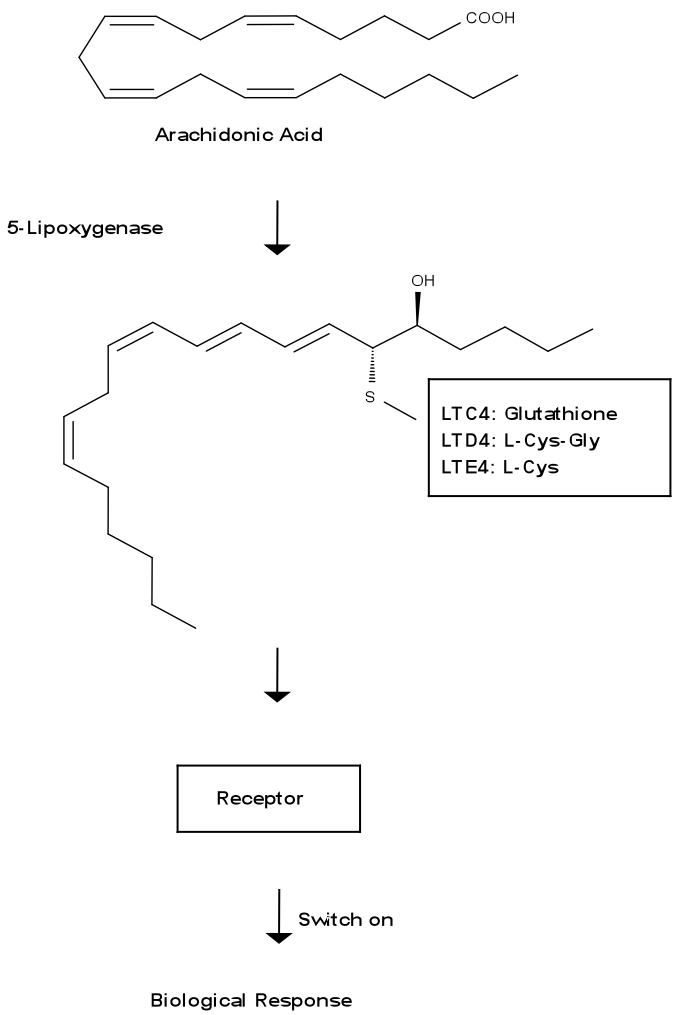
Treatment of acute symptoms is usually with an inhaled short-acting β-2 agonist (such as salbutamol) and oral corticosteroids. In very severe cases, intravenous corticosteroids, magnesium sulfate, and hospitalization may be required. Symptoms can be prevented by avoiding triggers, such as allergens and irritants, and by the use of inhaled corticosteroids. LABAs or antileukotriene agents (arachidonate 5-lipoxygenase inhibitors or LTD_4 antagonists) may be used in addition to inhaled corticosteroids, if asthma symptoms remain uncontrolled. As described above, various kinds of medications and treatments are used according to the symptoms presented in asthma.

Concept of LT antagonist discovery:[5] Oxidative metabolism of AA produces LTA_4, which is further converted to peptide LTC_4 by glutathione. Degradation of the peptide moiety of LTC_4 produces LTD_4, further degradation of which produces LTE_4. It is well known that LTs, consisting of LTC_4, LTD_4 and LTE_4, bind to the receptor to induce asthmatic response (Scheme 5). Thus, 5-lipoxygenase and LT receptors are considered to be promising drug discovery targets. Based on the information described above, many LT receptor antagonists and 5-lipoxygenase inhibitors have been evaluated as drug candidates. Among those tested, some LT receptor antagonists have been discovered to be promising drug candidates. On the other hand, it has been difficult to find a drug-like compound as an inhibitor of 5-lipoxygenase, mainly because of excessive lipophilicity.

Launched LTD_4 antagonists

Currently marketed LT receptor antagonists are described below because of their great benefit to clinical practice. Discovery of LT receptor antagonists in the AA cascade has led to great interest in this field. Among the clinically evaluated products, ONON, Singulair and Accolate are products which have been eventually marketed.

a) ONON™ (Pranlukast), Singulair™ (Montelukast) and Accolate™ (Zafirlukast) have been well known as LTD_4 receptor antagonists, and were launched as clinically useful drugs for the treatment of asthma. Administered orally twice, once, and twice daily respectively, they are LTD_4 antagonists that block the action of LTD_4 (and secondary ligands LTC_4 and LTE_4) by binding to the LT (cysteinyl leukotriene: CysLT) receptors: viz., the two subtypes $CysLT_1$ (in the lung) and $CysLT_2$ (in the bronchial tubes) receptors. It is of great interest that these three antagonists selectively block the subtype $CysLT_1$ receptor. They are orally active in suppression of bronchoconstriction, increased permeability of blood vessel, edema, hypersensitivity of mucous membrane, and other conditions accompanied by bronchial asthma and/or allergic rhinitis. These drugs are effective for the prevention of asthmatic attacks, although they are not effective in suppressing ongoing asthma.



Scheme 5: Mechanism of action of LTs *via* LT receptor

Because of their specific mechanism of action, they do not interact with other asthma medications such as theophylline. **ONON™ (Capsule: 450 mg/day), Singulair™ (Tablet: 10 mg/day)**, **Accolate™ (Tablet: 20~40 mg/day)** are marketed by Ono, Merck and Astra Zeneca, respectively. Their chemical structures and trade names (generic names) are shown below (Fig. 1):

b) Indications: Adult bronchial asthma, allergic rhinitis (ONON™ capsule: 450 mg/day; Singulair™ tablet: 10 mg/day; Accolate™ tablet: 20~40 mg/day) and infant bronchial asthma, allergic rhinitis (ONON™ dry syrup: 3.5~10 mg/day; Singulair™ chewable tablet 4~5 mg/day; Accolate™ should be used with extreme caution in CHILDREN younger than 5 years old; safety and effectiveness in these children have not been confirmed).

c) Adverse drug reactions (ADRs)

i) ONON™: Side-effects include headache, abdominal or stomach pain, cough, dental pain, dizziness, fever, heartburn, skin rash, stuffy nose, weakness or unusual tiredness. The above-described ADRs do not always cover all the side-effects. If patients have symptoms other than those described above, they should immediately go and see a physician or pharmacist.

ii) Singulair™: Get emergency medical help if Singulair-treated patients show signs of an allergic reaction: e.g. hives, difficulty in breathing, or swelling of face, lips, tongue, or throat. A physician should be notified immediately if patients experience unusual changes in mood or behavior, skin rashes, bruising, severe tingling, numbness, pain, muscle weakness, ear pain, swelling, or warm, severe skin reactions, fever, sore throat, swelling of face or tongue, burning sensation of eyes, or skin pain, followed by red or purple skin rashes that spread (especially to the face or upper body) and cause blistering and peeling.

Common Singulair side-effects may include: Stomachache, diarrhea, fever or other flu-like symptoms, cold symptoms such as stuffy nose, sinus pain, cough, sore throat, headache, bed-wetting or loss of bladder control in children.

iii) Accolate™: Diarrhea, headache, mild stomach pain, and nausea are the common symptoms. Patients should seek medical attention right away if any of these severe side-effects occurs: Severe allergic reactions (e.g. rashes; blisters; hives; itching; difficulty in breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue;

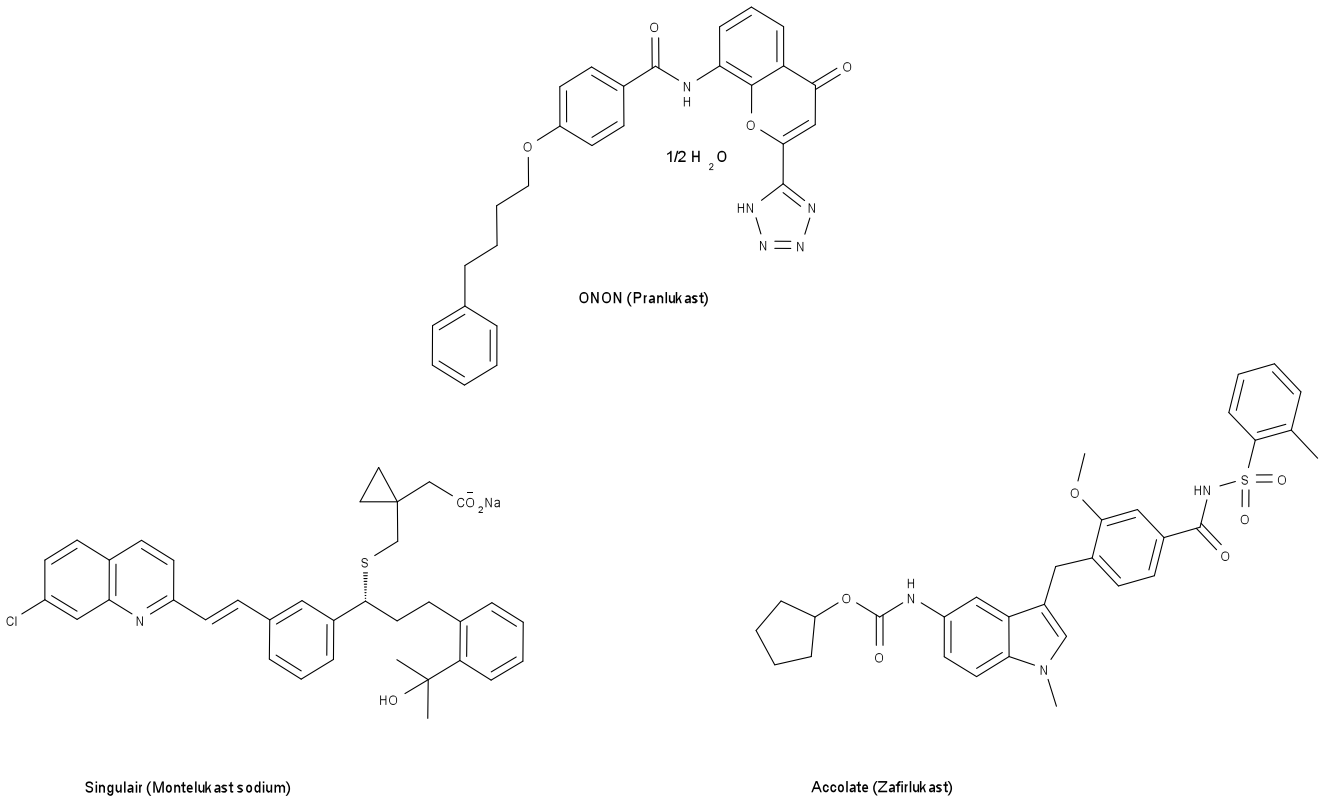


Fig. 1: Structures of launched LTD_4 antagonists

unusual hoarseness); burning, numbness, or tingling of the skin, hands, or feet; chest pain; fever, chills, or persistent sore throat; joint or muscle aches and pains; mental or mood changes (e.g. agitation, aggression, hostility, anxiety, depression, severe or persistent trouble sleeping, strange dreams, sleepwalking, tremor, hallucinations, restlessness, irritability, any unusual change in behavior); first-time or worsening shortness of breath or other breathing problems; suicidal thoughts or actions; swelling of the hands, ankles, or feet; symptoms of liver problems (e.g. yellowing of the skin or eyes, dark urine, flu-like symptoms, itching, loss of appetite, persistent nausea or stomach pain, unusual tiredness); unusual bruising or bleeding; and unusual sinus pain or swelling.

Special warning: A letter was submitted to the FDA by Zeneca Pharmaceuticals on July 22, 1997, notifying them of a change in product labeling that included the following potential reactions in patients undergoing a dosage reduction of oral steroids and who were currently taking Accolate™: Precautions-eosinophilic conditions. Reduction of the oral steroid dose, in some patients on Accolate therapy, has been followed in rare cases by the occurrence of eosinophilia, vasculitic rash, worsening pulmonary symptoms, cardiac complications, and/or neuropathy sometimes presenting as Churg-Strauss syndrome (a systemic eosinophilic vasculitis). Although a causal relationship with Accolate has not been established, caution is required when oral steroid reduction is being considered.

iv) Contraindications

These drugs are contraindicated for patients with known sensitivity to these drugs, pregnant women and nursing mothers. Patients with known aspirin sensitivity should be advised to continue to avoid aspirin and nonsteroidal anti-inflammatory agents while taking leukotriene antagonists.

v) Drug interactions

a) Coadministration of ONON™ with drugs such as itraconazole, erythromycin etc., which inhibit CYP3A4, may result in an increase in the concentration of these drugs because ONON is reported to inhibit CYP3A4 in in vitro and in vivo experiments.

b) No dose adjustment is needed when **Singulair™** is coadministered with theophylline, prednisone, prednisolone, oral contraceptives, terfenadine, digoxin, warfarin, thyroid hormones, sedative hypnotics, non-steroidal anti-inflammatory agents, benzodiazepines, decongestants, or hepatic cytochrome P450 (CYP) enzyme inducers (a large superfamily of enzymes with catalyze the oxidation of organic substances).

c) Coadministration of **Accolate™** with warfarin results in a clinically significant increase in prothrombin time and could result in bleeding. Prothrombin times should be monitored closely and anticoagulant therapy adjusted accordingly.

Since the discovery of the naturally occurring LTs, the inhibition of their biosynthesis and the antagonism of LTs at their receptors have been the objects of intense scientific investigations, with the expectation that such agents would be of medical value for the treatment of allergic asthma and other immediate hypersensitivity diseases. LT antagonists (ONON™, Singulair™ and Accolate™) and 5-lipoxygenase inhibitor (Zileuton™) were discovered from the 5-lipoxygenase pathway.

Competing Interests

Authors have declared that no competing interests exist.

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