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LEUKOTRIENE RECEPTOR ANTAGONISTS AS POTENTIAL THERAPEUTIC AGENTS

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INTRODUCTION

Leukotrienes are a family of bioactive lipids that have a constellation of pharmacologic effects on respiratory, cardiovascular, and gastrointestinal systems. They produce smooth muscle spasm (1, 2), cause myocardial depression (3), increase vascular permeability (1, 4) enhance mucous production (5), decrease mucociliary transport (6), and have the chemotactic property to attract leukocytes to a site of cellular injury (7, 8). The overwhelming evidence suggests that leukotriene synthesis and subsequent release in experimental animals and in humans are associated with certain pathophysiological events. Disorders such as asthma, adult respiratory distress syndrome, chronic bronchitis, cystic fibrosis, septic shock, psoriasis, inflammatory bowel disease, and myocardial ischemia have all been reported to be associated with increased levels of leukotrienes (9–15). Little is known about the physiological role, if any, of these C_{20} fatty acids but such information appears on the horizon. For example, recent studies have explored participation of leukotrienes in the fine tuning of the immune system (16). In addition, early evidence suggests a neuromodulatory role of leukotrienes (17, 18) and other lipoxygenase metabolites in the central nervous system.

Membrane phospholipids are the major source of arachidonic acid from which prostaglandins, thromboxanes, and leukotrienes are derived (Figure 1). Activation of phospholipase A_2, the enzyme that cleaves arachidonic acid from the two position of the phospholipid molecule, occurs as a consequence of anaphylactoid antigen-antibody reactions, by a variety of chemicals, and in response to cell injury. Leukotrienes are formed de novo by action of a

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5-lipoxygenase enzyme on newly released arachidonic acid through an intermediate, 5-hydroperoxyeicosatetranoic acid (5-HPETE). The first leukotriene (LT) to appear in the cascade, LTA_4, is short-lived, and is rapidly converted to either LTB_4 or LTC_4 by LTA_4 hydrolase or glutathione-S-transferase, respectively. LTC_4 and its cysteinyl leukotriene congeners, LTD_4 and LTE_4 (Figure 2), are collectively known as slow reacting substance of anaphylaxis (SRS-A). LTD_4 is formed from LTC_4 by γ-glutamyl-transpeptidase and subsequently converted to LTE_4 by aminopeptidase deglycination. This triad has created an interesting dialogue among investigators, on which of the three, if any, is more important in pathologic or physiologic events. This is reminiscent of the controversy of many years ago with another interesting biological threesome, dopamine, norepinephrine, and epinephrine. The lesson learned with the catecholamines will likely be relearned with the cysteinyl leukotrienes. Probably all three leukotrienes will eventually be shown to be important in their own right and to have their own pharmacologic receptors, and drugs will be developed to selectively antagonize their respective pharmacologic activities.

The number of publications describing various aspects of leukotriene pharmacology is growing at a meteoric rate. In addition, new molecules capable of selectively antagonizing leukotrienes are becoming commonplace. The intention of this article is not to exhaustively review this area of research, but to impress upon the reader that understanding the mechanism of action of these eicosanoids together with the successful development of their corre-

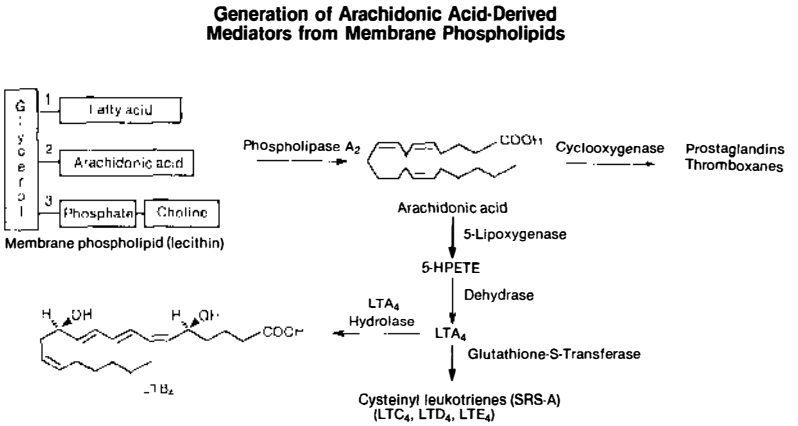

Figure 1 Schematic representation of leukotriene synthesis from membrane phospholipids via arachidonic acid. Note that arachidonic acid is the precursor for thromboxanes and prostaglandins as well as leukotrienes.

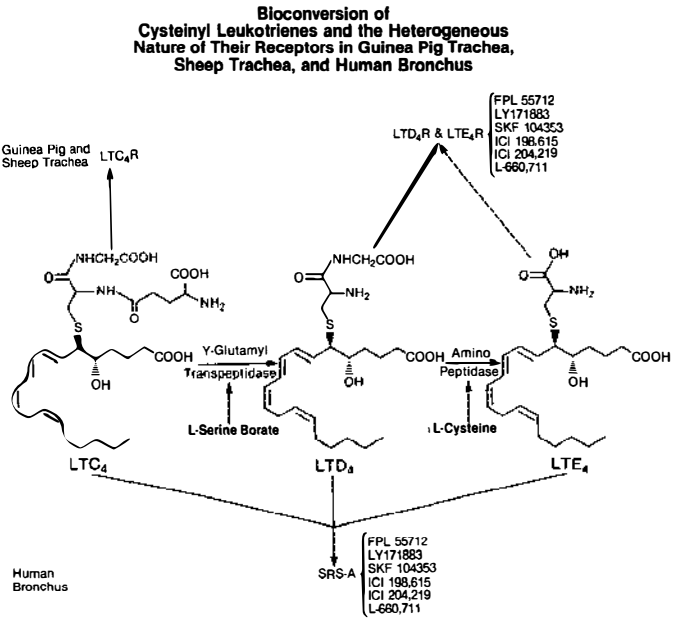

Figure 2 This illustration depicts the nature of cysteinyl leukotriene receptors, being heterogeneous in guinea pig and sheep trachea with distinct LTC_4 and LTD_4/LTE_4 receptors whereas in human bronchus the three cysteinyl leukotrienes appear to activate an "SRS-A" receptor. Leukotriene-mediated responses in sheep and guinea pig differ in that LTE_4 is not an agonist in sheep trachea.

sponding receptor antagonists potentially holds the key to new and more versatile therapeutic agents.

Pharmacology of Leukotrienes on Airway Smooth Muscle

Most work defining the pharmacology of leukotrienes on airway smooth muscle has been performed either in guinea pig or on tissues isolated from this species. Leukotrienes are the most potent constrictors of guinea pig airway smooth muscle yet described; LTC_4 and LTD_4 are 1000 times more potent than histamine. In guinea pig parenchymal strips, LTA_4 and LTB_4 are at least an order of magnitude more potent than histamine (19–22). Efficacy of the leukotrienes, however, is only 70–90% of that produced by histamine or carbachol. In isolated airways from guinea pigs, the rank order of potency is $LTC_4 = LTD_4 > LTE_4$ (23). Similar studies with isolated human intralobar smooth muscle found the cysteinyl leukotrienes equipotent (24), whereas suprafused human bronchus studies showed a rank order of potency of $LTC_4 = LTD_4 > LTE_4$ (25). This correlates more closely with their in vivo potency and suggests that LTE_4 is a metabolite destined for further inactiva-

tion. Airway smooth muscle of most laboratory species, in contrast to guinea pigs and humans, is much less responsive to the leukotrienes (22, 26–28).

The contractile activities of the cysteinyl leukotrienes on smooth muscle result from direct activation of membrane receptors, or indirectly through release of secondary mediators (20, 21, 26). The modest contractile activity of LTB_4, a noncysteinyl containing dihydroxy leukotriene, is mediated primarily through indirect mechanisms in lung parenchymal strips (20). Several investigators have demonstrated that the indirect mechanisms involve release of cyclooxygenase products from airway smooth muscle that in turn modulate contractile responses (20, 21, 26, 27, 29, 30). Interestingly, the prostanoid contractile component of the leukotriene response is more evident using suprafused techniques, whereas the prostanoid relaxant component is more prominent using organ bath techniques (20, 26, 29). Treating with indomethacin, a cyclooxygenase inhibitor, would result in opposite effects on the leukotriene responses, depending on the experimental methods employed. Thus, the necessity to clearly define experimental procedures when studying the leukotrienes is of paramount importance. LTB_4- and LTC_4-induced contractions of isolated lung parenchyma are not blocked by receptor antagonists of acetylcholine, histamine (H_1), norepinephrine, or serotonin, which indicates that indirect actions of the leukotrienes are not mediated by release of these non-prostanoid agonists (20, 26, 31, 32).

Biochemical studies demonstrated that direct and indirect actions of the cysteinyl leukotrienes involve specific receptors. Leukotrienes reversibly bind to these tissue macromolecules. This process is saturable and shows stereochemical specificity (33–37). Pharmacologic activities of the leukotrienes are concentration-dependent, stereochemical specificity is required, and these effects can be antagonized by selective receptor antagonists (38–42). Like many other naturally occurring agonists, the leukotrienes act on a heterogeneous population of receptors, (43–45). This heterogeneity occurs between species, among different organs of the same species, and even within the same tissue, although some commonality does exist. For example, LTE_4 is an effective ligand able to displace radio-labeled LTD_4 as well as LTE_4 from lung membranes preparations, indicating that receptors for LTD_4 and LTE_4 may be similar (33, 34, 46). Functional studies also suggest that LTD_4 and LTE_4 share common receptor subsets since the contractile responses of these mediators are antagonized by similar agents despite slightly different potencies (42, 44, 47). In contrast, both binding and functional studies have demonstrated the uniqueness of the LTC_4 receptor. Binding studies with ^3H–LTC_4 in numerous tissues have presented data consistent with the existence of a specific LTC_4 receptor (48, 49). Good correlations between specific agonist binding and pharmacologic effects with LTC_4 have not been obtained (49) and bring into question the functional significance of an LTC_4

receptor. This can be explained by the ability of LTC_4 to bind glutathione-S-transferase (50, 51). The wide distribution of this enzyme in vivo has led to a higher than expected number of LTC_4 receptors. A recent report on autoradiographic localization of leukotrienes in guinea pig lung confirms not only the distinct receptor hypothesis for LTC_4 and LTD_4, but also supports the abundant binding characteristics of LTC_4 (52).

Strong functional evidence for a separate LTC_4 receptor was reported by Snyder & Krell (45) and later confirmed by Weichman & Tucker (53) using guinea pig trachea. L-Serine borate was used to block the bioconversion of LTC_4 to LTD_4 (Figure 2) (23), which rendered the leukotriene receptor antagonist FPL 55712 (41) inactive in blocking contractions to LTC_4. In contrast, LTD_4-induced responses were effectively blocked by FPL 55712 in the presence or absence of L-serine borate. Similar results were recently obtained in trachea isolated from sheep (54). Studies on intralobar airways resected from individuals with lung carcinomas have not revealed evidence for multiple leukotriene receptors (24). In this later study, FPL 55712 was an effective competitive inhibitor of the contractile activities of LTC_4 and LTD_4, with similar pK_B values for each agonist independent of the presence of metabolic inhibitors. In a more recent study by Buckner et al (55), ICI 204219 antagonized the contractile responses of all three cysteinyl leukotrienes on human airways with equal potency. One explanation is that human airways may contain only a single class of leukotriene receptors similar to the LTD_4/LTE_4 receptors identified in guinea pig trachea. Inhibition of the metabolic conversion of LTC_4 to LTD_4 by L-serine borate was not measured directly in human airways (24). Circumstantial evidence based on the ability of L-serine borate to block metabolic conversion of LTC_4 to LTD_4 in chopped human lung (56) and guinea pig trachea (23) was used to support their findings. The possibility exists that L-serine borate is not an effective inhibitor of γ-glutamyl-transpeptidase in human intralobar airways especially since the rate of conversion of LTC_4 to LTD_4 occurs more rapidly in human than guinea pig lung or trachea (23, 56). FPL 55712 may therefore have inhibited LTC_4-induced contractions due to the conversion of LTC_4 to LTD_4.

Different Structural Types of Leukotriene Receptor Antagonists

Since leukotrienes are believed to play a pathophysiological role in several disease states, the need for a leukotriene receptor antagonist would be fundamental to the improvement of the pathology. Three distinct approaches have been used in their design: (*a*) analogs of FPL 55712 which contain the acetophenone moiety; (*b*) analogs of the natural agonists; and (*c*) novel chemicals that do not fall into either of these two categories.

FPL 55712 (41) was the first leukotriene receptor antagonist identified and

for six years was used as an SRS-A antagonist. But its poor bioavailability and short half-life limited its use in various animal models (57, 58), and precluded its development as a therapeutic agent. The elucidation of the structural components of SRS-A (59, 60) brought the acceptance of FPL 55712 as an LTD_4/LTE_4 receptor antagonist. FPL 55712 played an essential role in defining the pharmacology of the cysteinyl leukotrienes. LY171883, LY163443, L-649, 923, L-648,051, and YM-16638 (Figure 3) are a few acetophenone-containing analogs designed to overcome the limitations that beset FPL 55712. For example, LY171883, LY163443 and L-649,923 had similar potency to FPL 55712 but were able to block leukotriene-mediated bronchoconstriction in guinea pigs following oral administration at 3–10 mg/kg (42, 61, 62).

SKF 104353 (Figure 4) is the leading example of an antagonist whose structure was derived from the natural agonists. SKF 104353 has a pA_2 of 8.6 against LTD_4-induced contractions of guinea-pig trachea (63), which makes it more potent than those in the FPL 55712 series. One important obstacle in developing such compounds is effective affinity for the receptor with total loss of intrinsic agonist activity or efficacy. An active antagonist in various animal models could conceivably show agonist activity in man, especially in the asthmatic where airway smooth muscle is hyperresponsive. The question remains whether SKF 104353 has completely overcome this obstacle.

The third class of antagonists is comprised of a group of structurally unrelated chemicals. ICI 198,615, ICI 204,219, Wy-48,252, ONO RS 411, and L-660,711 (Figure 4) are examples of compounds in this class. ICI

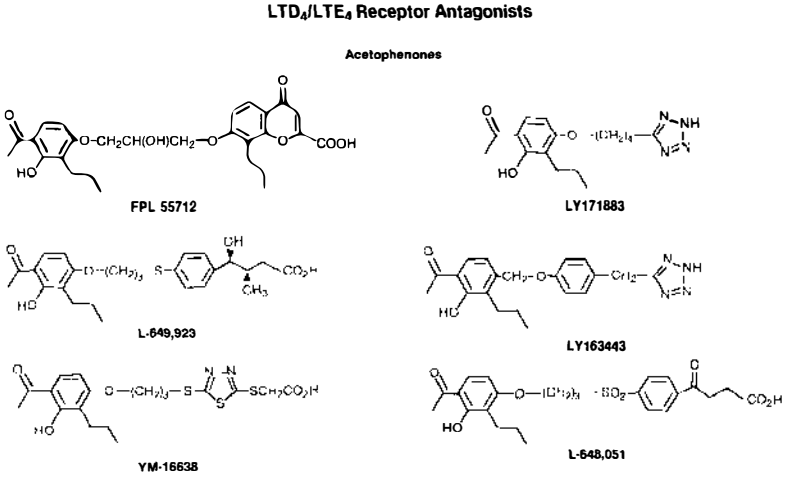

Figure 3 Chemical structures of LTD_4/LTE_4 receptor antagonists: acetophenone containing analogs.

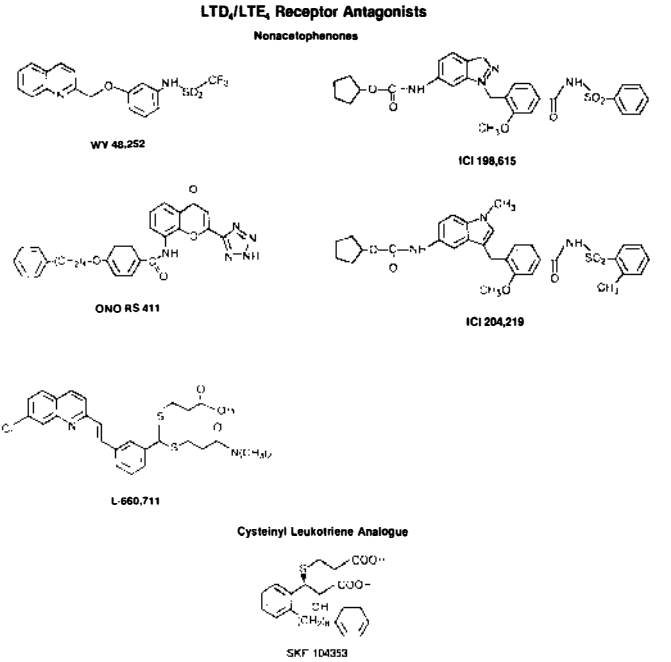

Figure 4 Chemical structures of LTD_4/LTE_4 receptor antagonists: nonacetophenone and cysteinyl leukotriene analogs.

204,219 has a pK_B of 9.6 against LTD_4-induced contractions of guinea-pig trachea (55) and is the most potent antagonist reported to date in clinical trial. Activity against LTC_4-induced contractions is lacking.

Fundamental Characteristics of Leukotriene Receptor Antagonists

In developing a leukotriene receptor antagonist, a compound should possess certain basic properties to assure its progression to clinical trials in a therapeutic area. These would include potency or affinity for the receptor, lack of intrinsic activity, receptor selectivity, adequate duration of action, bioavailability, efficacy in various animal models of the disorder, and lack of toxicity. The second generation compounds, LY171883 and L-649,923, overcame the limitations that besieged FPL 55712 but did not dramatically improve potency. L-648,051 could also be considered in this class but because of poor oral bioavailability, its potential use was restricted to an aerosol therapeutic agent (64). Clinical trials have been initiated and progressed farthest with LY171883. The advent of the third generation of leukotriene antagonists, as represented by SKF 104353, ICI 198,615, ICI

204,219 and L-660,711, brought marked improvement in potency. These are 1–3 orders of magnitude more potent than LY171883. SKF 104353 and ICI 204,219 are in Phase II and Phase I of clinical evaluation, respectively. The former compound, like L-648,051, appears destined for use as an aerosol (63). The pharmacology of L-660,711 was recently described (65, 66) and is now in the early stages of development. The enhanced potencies of these drugs may improve their potential for therapeutic efficacy in the treatment of leukotriene-related diseases.

Selectivity of an antagonist for a leukotriene receptor is of vital importance. The compound should have high affinity for the receptor and little or no affinity toward nonleukotriene receptors such as those for norepinephrine, histamine, serotonin, acetylcholine, thromboxane A_2 and prostaglandin E_2 and D_2. The second generation leukotriene antagonists had approximately 10–50 fold more affinity for the LTD_4/LTE_4 receptors (42, 61, 62). In contrast, SKF 104353, ICI 198,615, ICI 204,219 and L-660,711 display an approximately 1,000- to 16,000-fold preference for LTD_4/LTE_4 receptors (47, 63, 65, 67). In addition to these properties, LY171883 initially was reported to be a weak inhibitor of phosphodiesterase (42), which could have contributed to its pharmacologic activity. However, a number of findings argue against this possibility, not the lease that it is about 200–fold more potent as a leukotriene antagonist than as an inhibitor of phosphodiesterase. Recent studies by Rinkema et al (68) compared LY171883, isobutylmethylxanthine (IBMX), and theophylline as LTE_4 receptor antagonists and as inhibitors of phosphodiesterase in a number of in vitro and in vivo test systems. The ability of these three agents to potentiate the actions of isoproterenol was judged a reflection of inhibition of phosphodiesterase. LY171883 functioned primarily as an LTE_4 receptor antagonist whereas the actions of IBMX and theophylline appeared to reflect inhibition of phosphodiesterase.

All leukotriene receptor antagonists identified to date have been selective in inhibiting the LTD_4/LTE_4 receptor as defined in animal models. All are virtually devoid of activity toward the LTC_4 receptor. Would an agent that also has activity against the LTC_4 receptor be advantageous in treating either the early or late phase of asthma? In sheep, considerable experimental evidence has implicated cysteinyl leukotrienes as major mediators of antigen-induced late phase bronchospasm (69). In this animal model, the late phase reaction was only marginally reduced by metaproterenol, a beta receptor agonist (70) showing marked similarity to the human syndrome (71–74). Interestingly, in guinea pig trachea, contractions mediated by LTC_4 receptors were less sensitive to the relaxant effects of salbutamol, another beta receptor agonist, than those mediated by LTD_4/LTE_4 receptors (75). These observations might suggest that late phase asthmatic responses are mediated, in part, through activation of LTC_4 receptors as well as LTD_4/LTE_4 receptors. On the

other hand, a group of LTD_4/LTE_4 receptor antagonists, including LY171883, abolished the late bronchospasm in allergic sheep (69, 76). Therefore, an LTC_4 receptor antagonist may not be necessary if the human late phase reaction is similar to that seen in allergic sheep. Recently, cysteinyl leukotriene receptors in isolated tracheal smooth muscle from sheep were characterized (54). LTE_4 was virtually devoid of agonist activity in sheep trachea. LTD_4-induced responses were antagonized by an LTD_4/LTE_4 receptor antagonist, YM-16638. LTC_4-induced contractions, in the presence of L-serine borate, were not antagonized by YM-16638. Evidence that L-serine borate effectively blocked conversion of LTC_4 to LTD_4 was noted by the leftward shift, approximately 60-fold, in the LTC_4 concentration-response curves. These results indicate that leukotriene receptors in sheep trachea consist of LTC_4 and LTD_4 receptors that appear functionally related to LTC_4 and LTD_4/LTE_4 receptors in guinea pig trachea, respectively.

Cysteinyl leukotriene receptors in human airways, unlike those in sheep and guinea pig, appear to be more homogeneous and are sensitive to the actions of LTD_4/LTE_4 receptor antagonists (Figure 2) (24, 55, 62, 63). Homogeneity was defined in samples of airways obtained from individuals who do not possess hyperresponsive airways, i.e. nonasthmatics. The possibility exists that distinct LTC_4 receptors may be elaborated in the asthmatic and thereby contribute to hyperresponsive airways. Alternatively, the treatment regimes and anesthetic protocols used in these lung carcinoma patients may have masked the responses normally mediated by LTC_4 receptors. With samples of resected human lung, responses of the three cysteinyl leukotrienes appear to be acting through LTD_4/LTE_4 receptors based on their sensitivity toward existing leukotriene receptor antagonists. Therefore, testing the hypothesis of functional LTC_4 receptors in human airways cannot be completed until samples of intralobar airways obtained from asthmatics are tested in vitro or until LTC_4 receptor antagonists have been developed and tested in man. Sensitivity toward LTC_4 as well as LTD_4/LTE_4 receptors might prove beneficial for an ideal leukotriene receptor antagonist. This would ultimately bring us full circle in the development of an SRS-A receptor antagonist that was initiated 15 years ago with FPL 55712.

Another product of the arachidonic acid cascade via 5-lipoxygenase pathway is LTB_4 (Figure 1). This member of the leukotriene family has potent chemotactic properties as well as the ability to degranulate human neutrophils (77). Effects of LTB_4 are mediated through separate, distinct, stereospecific, high and low affinity receptor sites on the leukocyte cell surface (77). LTB_4 has been associated with airway hyperresponsiveness in dog (78) and with increased levels in lung lavages from humans with severe pulmonary dysfunction (79). Aerosol administration of LTB_4 to conscious guinea pig has resulted in increased numbers of neutrophils and eosinophils in mucosal/

submucosal regions of trachea and bronchi and altered airway responsiveness (80). Eosinophils isolated from patients with asthma demonstrated enhanced 5-lipoxygenase activity, producing three times more LTC_4 per cell than eosinophils isolated from normal subjects (81, 82). Furthermore, asthmatic patients may have a larger proportion of lower density eosinophils compared to nonasthmatics (83, 84). In vitro studies have demonstrated that granulocyte-monocyte colony-stimulating factor produces a phenotypic change from normodense to hypodense eosinophils resulting in an increased capacity to generate LTC_4 (85). Thus, blocking the actions of LTB_4 at the receptor level and thereby preventing recruitment of neutrophils and eosinophils should reduce some of the symptoms associated with inflammatory pulmonary disease.

Unlike LTD_4/LTE_4 receptor antagonists, identification of LTB_4 receptor antagonists has progressed at a slow pace. SM-9064, an analog of LTB_4, was the first to be described in the literature (86) (Figure 5). LTB_4-induced chemotaxis of rat polymorphonuclear leukocytes was inhibited by SM-9064 with an IC_{50} of 0.16 μM. In another series of LTB_4 analogs, U-75302 (Figure 5) appeared to be the most active, reducing ^{3}H-LTB_4 binding when tested at 1.0 μM (87). This study did not include functional assays so it is difficult to differentiate agonist from antagonist activities under the conditions employed. Recently, LY223982, a benzophenone analog, and LY255283, (Figure 5) an acetophenone analog, were reported to be potent inhibitors (IC_{50} = 12 and 87 nM respectively) of ^{3}H-LTB_4 binding to human neutrophils (88–90). Functional studies demonstrated that 0.1 μM of either compound inhibited LTB_4-induced aggregation of guinea pig neutrophils by 50%. These or similar compounds will be useful in determining the role of LTB_4 in various disease states and such properties might be useful additions to the profile of an ideal leukotriene receptor antagonist.

With the enhanced elaboration of 5-lipoxygenase products from inflammatory cells in asthmatics (83, 84) and with the unknown role of LTC_4 in this disease state, the ultimate leukotriene receptor antagonist may need to possess potent, selective inhibition against 5-lipoxygenase enzymes. Wy-48,252 contains both these properties (Figure 4) (91). Relative to ICI 198,615 (47), this compound is a weak LTD_4/LTE_4 antagonist, pK_B = 7.4, but its activity against 5-lipoxygenase (IC_{50} = 4.6 μM) may prove advantageous. The combined action of these activities makes Wy-48,252 effective in various animal models (91) that may have been less effective with either single mechanism of action. This dual action compound would not only inhibit the synthesis of LTB_4 and of LTC_4 but perhaps more importantly could also limit the formation of lipid-derived oxygen radicals which is thought to occur during the synthesis of 5-LO products. These reactive molecules are believed to be responsible for various types of cellular injuries associated with the inflammatory disease process.

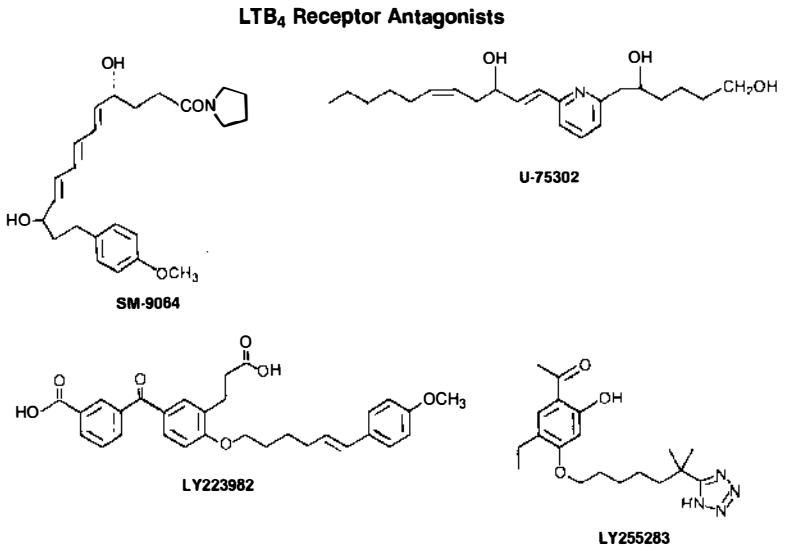

Figure 5 Chemical structures of LTB_4 receptor antagonists.

Human Pharmacology of Leukotrienes

Even though LTB_4 and the cysteinyl leukotrienes are generated and released from a variety of cell types and have been shown to exert powerful pharmacologic actions in numerous test systems, there is only circumstantial evidence that these substances contribute to human disease. Some of the first studies with SRS-A by Brocklehurst (92), in isolated human bronchioles, had demonstrated the bronchospastic properties of this putative mediator. Six years later Herxheimer & Stresemann (93) aerosolized SRS-A into the lungs of nine asthmatic subjects. These individuals experienced a reduction in vital capacity. Interestingly, no effect was seen in four nonasthmatic volunteers exposed to SRS-A for 12 min. These early studies helped formulate the hypothesis that SRS-A played a pivotal role in human asthma.

The postulated involvement of leukotrienes in human diseases has received significant support during the past few years. Leukotrienes were shown to exert effects in humans similar to those observed in laboratory animals. In addition, elevated levels of leukotrienes have been found in selected body fluids from individuals afflicted with a variety of disorders (94–97).

The initial demonstration that chemically pure LTC_4 and LTD_4 were bronchospastic in humans came from Holroyde et al (98) who aerosolized

solutions of these anaphylactic mediators into the lungs of two nonatopic volunteers. Subsequently, a plethora of studies found both LTC_4 and LTD_4 to be among the most potent bronchoconstrictor substances known (see reviews by Drazen, (2); and Drazen & Austen, (99)). Davidson et al (100) administered LTE_4 by aerosol to normal and asthmatic subjects. Although LTE_4 was the least potent bronchoconstrictor of the three cysteinyl leukotrienes in normal subjects, it was still 39 times more potent than histamine. To date, after many leukotriene aerosol challenges, the high potency of the cysteinyl leukotrienes relative to agonists such as histamine and methacholine has been firmly established, a fact that befits their role in obstructive airway disease. The safety with which these inhalations have been performed together with the possible role of leukotrienes in the asthma syndrome opens debate as to whether chemically pure cysteinyl leukotrienes might prove useful as diagnostic tools for classifying individuals with reversible airway disease. Leukotriene inhalation in volunteers would also prove useful in early clinical development of novel leukotriene receptor antagonists. They could help establish efficacy of potential new agents on the human respiratory system and aid in selecting appropriate doses to use in advanced studies in patients with active disease. Furthermore, specificity of new agents for human leukotriene receptors in vivo would be readily ascertained.

Additional clinical investigations with the leukotrienes have expanded the understanding of their human pharmacology beyond that obtainable by direct inhalation into the lungs. Bisgaard et al (101) studied the influence of LTD_4 on nasal mucosal blood flow, nasal airway resistance, and nasal secretion in 34 persons. Topical LTD_4 increased nasal mucosal blood flow reaching a maximum after 8 min, an effect equipotent with histamine. Nasal airway resistance also increased in a dose-dependent manner after LTD_4. Itching and sneezing was associated with topical application of histamine, as was an increase in the amount of nasal secretions. In contrast, LTD_4 did not increase nasal secretions nor was itching and sneezing reported. Similar conclusions with LTC_4 and histamine were also reported by Miadonna et al (102). These observations using direct application of LTC_4, LTD_4, and histamine to the nasal mucosa and those reporting release of numerous bioactive molecules from the nose after antigen challenge (103) point strongly to a role for the leukotrienes, in conjunction with other mediators, in allergic rhinitis.

Animal studies have repeatedly demonstrated the ability of LTD_4 to increase vascular permeability leading to leakage of fluid from the vascular space (1, 4). Soter et al (104) examined the consequence of intracutaneous administration of LTB_4, LTC_4, LTD_4, and LTE_4 in human skin. LTB_4 caused a transient wheal and flare, followed in three to four hours with a dermal infiltration comprised mostly of neutrophils. The cysteinyl leukotrienes elicited a more long lasting erythema and wheal formation. More recent ex-

periments by Bisgaard (105) demonstrated increased blood flow rate in human skin after intracutaneous administration of LTD_4 or histamine. As with nasal mucosal blood flow (101), the two mediators were equipotent on the cutaneous vascular bed. Greenberger et al (106) sought to compare the dermal effects of intracutaneous LTD_4 or histamine in normal volunteers and patients with asthma or allergic rhinitis. The threshold doses of LTD_4 and histamine required to produce a characteristic wheal and flare did not differ between the groups: this suggests that atopy does not impart cutaneous hyperreactivity to these mediators.

Cysteinyl leukotrienes have profound effects on the cardiovascular system of laboratory animals (3, 14) and subhuman primates. In anesthetized adult male rhesus monkeys, Hahn & MacDonald (107) demonstrated that i.v. infusion of LTD_4 produced dose-related depression of myocardial contractility, stroke volume, and aortic blood flow in association with systemic and pulmonary vasoconstriction. These LTD_4-mediated effects were reduced by LY171883. Marone et al (108) provided evidence of a similar effect of LTC_4 in human subjects undergoing diagnostic cardiac catherization. A bolus i.v. injection of two nmoles of LTC_4 caused a fall in mean arterial pressure associated with a rise in heart rate. This was not accompanied by immediate changes in coronary blood flow or coronary vascular resistance. However, 10 min after LTC_4, coronary vascular resistance increased, followed by a return to baseline after an additional 10 min. LTC_4 administration also caused a rise in plasma levels of epinephrine and norepinephrine signalling a sympathoadrenergic discharge. In another section of the same study, the investigators gave three nmoles LTD_4 via intracoronary injection and obtained results similar to those described for LTC_4. Thus, whether in small laboratory animals, subhuman primates, or humans, cysteinyl leukotrienes have the capacity to produce marked cardiovascular dysfunction supporting a role for these molecules in the symptomatology of various forms of shock (12, 14, 109).

Human Pharmacologic Evaluation of Leukotriene Receptor Antagonists

The end product of basic pharmacologic investigations is development of novel therapeutic agents to treat human disease. Thus, it comes as no surprise that with evidence mounting for leukotriene involvement in a myriad of illnesses, much effort has gone into the development of leukotriene receptor antagonists. As indicated above, very potent drugs specific for LTD_4 and LTE_4 receptors are now available for clinical evaluation. The initial indication has been toward chronic treatment of asthma. As with all potential new therapies, success is measured in small steps, each vitally dependent on preceding studies.

Although long term animal toxicity caused its withdrawal from clinical trials, LY171883 has provided much of the available information on responses of human volunteers and asthmatic patients to an LTD_4/LTE_4 receptor antagonist. The first critical observation resulting from phase I trials indicated that presumptive block of LTD_4 and LTE_4 receptors in humans did not produce significant side effects: this suggests that LTD_4, LTE_4, and possibly even LTC_4 are not involved in major physiological processes (110). If they were, pharmacologic antagonism of leukotriene receptors would have resulted in an unacceptable side effect profile. An example is atropine, a muscarinic receptor antagonist that may cause tachycardia, dry mouth, a decrease in gastrointestinal motility, and blurring of vision as possible side effects. These actions are due to involvement of muscarinic cholinergic receptors in the physiologic function of the heart, salivary glands, gastrointestinal tract, and in accommodation of the eyes. During two early clinical experiences with L-649,923, patients experienced acute abdominal discomfort and watery diarrhea (111, 112). Neither group of investigators studying L-649,923 could rule out the possibility that these observations were related to blocking normal physiological effects of cysteinyl leukotrienes in humans. The profile of LY171883 in humans implies that these actions were specifically related to L-649,923 and not to leukotriene antagonists in general. Only additional studies with structurally unrelated leukotriene receptor antagonists will provide an unambiguous answer. A recent publication describing a phase I clinical study with L-648,051 (64,113), an analog of L-649,923, may have already provided insight into whether the latter antagonist disrupted a physiological action of the leukotrienes on the human gastrointestinal tract. L-648,051, in doses up to 70 mg administered i.v. to healthy volunteers, proved safe and well-tolerated. The only adverse event reported was a local irritation at the site of injection (113).

LY171883 (114) and L-649,923 (112) were evaluated as LTD_4 receptor antagonists in human volunteers who underwent an LTD_4 inhalation challenge before and after administration of 1 gram L-649,923 or up to 400 mg LY171883. L-649,923 produced an approximately four fold rightward shift of the LTD_4 dose-response curve; LY171883 caused a slightly greater effect. This relatively modest reduction in leukotriene-induced bronchospasm was somewhat surprising and reflected either insufficient potency of the compounds or perhaps significant differences, not currently appreciated, between cysteinyl leukotriene receptors in humans and those in experimental animals. Future studies in humans with more potent leukotriene receptor antagonists should differentiate between these two possibilities.

Three recently reported clinical studies with LY171883 point to the usefulness of LTD_4/LTE_4 receptor antagonists in the treatment of human asthma. The largest was carried out in 138 patients (115). Approximately half took LY171883 while the other group was maintained on placebo therapy. Patients

were permitted free access to inhaled bronchodilator in the form of metaproterenol, a beta receptor agonist. After six weeks, LY171883-treated patients showed objective improvement of their asthma as compared to the placebo group. Their ability to perform an FEV_1 (forced expiratory volume in one second) maneuver, an index of pulmonary function, improved during the course of treatment. Of great interest was the find that in those individuals most dependent on metaproterenol therapy, there was a dramatic reduction in beta receptor agonist usage.

Cold air or exercise challenge elicits bronchoconstriction in asthmatic subjects (116). The nature of the mediators participating in this response is presently unknown. However, with the advent of specific pharmacologic antagonists of the various mediators of anaphylaxis, investigators can begin systematically to determine the contribution individual mediators might make to this airway obstruction. Israel et al (117) reported results of a randomized, double blind, placebo-controlled trial in which LY171883 reduced bronchoconstriction induced by cold air challenge in asthmatics. Although not a large effect, it provides initial evidence for liberation of LTD_4 or LTE_4 in human lung during inhalation of cold air. Likewise, Shaker et al (118) treated ten exercise-induced asthmatics with LY171883 or placebo. These patients were characterized by showing a greater than 19% drop in FEV_1 following treadmill exercise. After 14 days of treatment, 5 individuals showed marked improvement in FEV_1, suggesting a role for LTD_4 or LTE_4 in their asthma; 4 patients did not improve and one individual failed to complete the protocol. The mixed results with LY171883 is puzzling but may illustrate the multifactorial nature of asthma. Again, we must stress that clinical testing of this type must be performed with a variety of leukotriene receptor antagonists to insure the validity of any conclusions that relate clinical efficacy of these drugs to their ability to block actions of the leukotrienes. Just as LY171883 gave some initial, promising data on the efficacy of leukotriene receptor antagonists in asthma, SKF 104353, ICI 204, 219, and L-660, 711 should further enlighten the role of leukotrienes in asthma and other disease states in which leukotrienes are believed to be involved.

COMMENTARY AND CONCLUSIONS

Numerous human disorders appear to be associated with endogenous generation of potent molecules termed mediators. A large number of these substances are derived from membrane phospholipids and are metabolites of arachidonic acid. Currently, a major effort is under way to develop potent, selective, and nontoxic drugs capable of antagonizing the actions of various mediators. A criticism frequently leveled against this research is that with the myriad of mediators produced in vivo during the active phase of an illness, a drug with activity against a single mediator would not be an effective therapeutic agent. The ideal agent would then be one with a broad spectrum,

either as an antagonist or as an inhibitor of synthesis or release of the mediators. This approach does not consider the probability that some of the newly released molecules result from the body's attempt to negate the ongoing assault. If this is so, then an appropriate course of action would be to pharmacologically antagonize only those substances known to be disruptive to the system. Furthermore, if one mediator stands out as a primary offender, then termination of its action should restore the status quo. Of course, it stands to reason that the most highly selective compounds tend to have the most favorable side effect profile.

Within the past decade, this area of research has gone from studying a lipid mediator, SRS-A, and a single SRS-A antagonist, FPL 55712, to our current situation represented by an in-depth understanding of eicosanoids and a variety of drugs capable of specifically reducing their biological effects. The possibility exists that some of these antagonists will be tomorrow's new therapies.

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