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POTENTIAL ANTI-INFLAMMATORY EFFECTS OF 5-LIPOXYGENASE INHIBITION — EXAMPLIFIED BY THE LEUKOTRIENE SYNTHESIS INHIBITOR BAY X 1005

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Leukotrienes have been identified in various pathophysiologies. The leukotrienes LTB_4 and LTC_4 are assigned to inflammation. 5-lipoxygenase inhibitors which inhibit the synthesis of LTA_4 being the precursor of both LTB_4 and LTC_4 appear to have only a limited antiinflammatory potential.
5-lipoxygenase inhibitors are represented by direct and indirect inhibitors, the latter competing with substrate transfer from the five-lipoxygenase activating protein (FLAP) to the 5-lipoxygenase enzyme. 5-lipoxygenase inhibition under experimental condition results in inhibition of edema formation, neutrophil infiltration, smooth muscle contraction after antigen challenge and prevention of early and late allergic reactions. Only in the cysteinyl-leukotriene-driven pathophysiology of allergic asthma and allergic rhinitis 5-lipoxygenase inhibition appears to provide symptomatic relief. Yet, the overall-antiinflammatory effect in man is far less than expected, but may be outweighed by the nearly total lack of any side effects of 5-lipoxygenase inhibition *per se*.

K e y w o r d s: *inflammation, leukotriene, 5-lipoxygenase, leukotriene synthesis inihibitor, BAY X 1005, allergic asthma.*

INTRODUCTION

Leukotrienes (LTB_4 and cysteinyl-leukotrienes: LTD_4, LTE_4) are important inflammatory mediators. They have been early on assigned to allergic asthma, firstly named slow-reacting substance of anaphylaxis (1). LTB_4 is an autocrine activator of neutrophil granulocytes (PMNL, polymorphonuclear leukocytes) having a similar profile like complement anaphylatoxin (C5a) and the cytokine interleukin 8 (IL-8) (2).

Cysteinyl-leukotrienes (CysLT) contract smooth muscle cells of small and large arteries and bronchial airways (1). They also activate endothelial cells to promote leukocyte adherence and invasion (3).

Anti-leukotriene agents

Leukotriene B_4 and the CysLT's are both generated *via* the 5-lipoxygenase (5-LOX) pathway. The 5-LOX is unique by its interaction with the Five Lipoxygenase Activating Protein (FLAP) which enhances its activity and increases its substrate specificity (4).

The LOI (5-lipoxygenase inhibitors) interfere with the 5-LOX enzyme directly whereas the leukotriene synthesis inhibitors (LSI) bind to FLAP and interfere with the substrate transfer and 5-LOX translocation (1, 5).

Both types of 5-LOX-inhibitors, the direct or indirect ones prevent the formation of the intermediate leukotriene LTA_4 (*Fig. 1*). LTA_4 is either

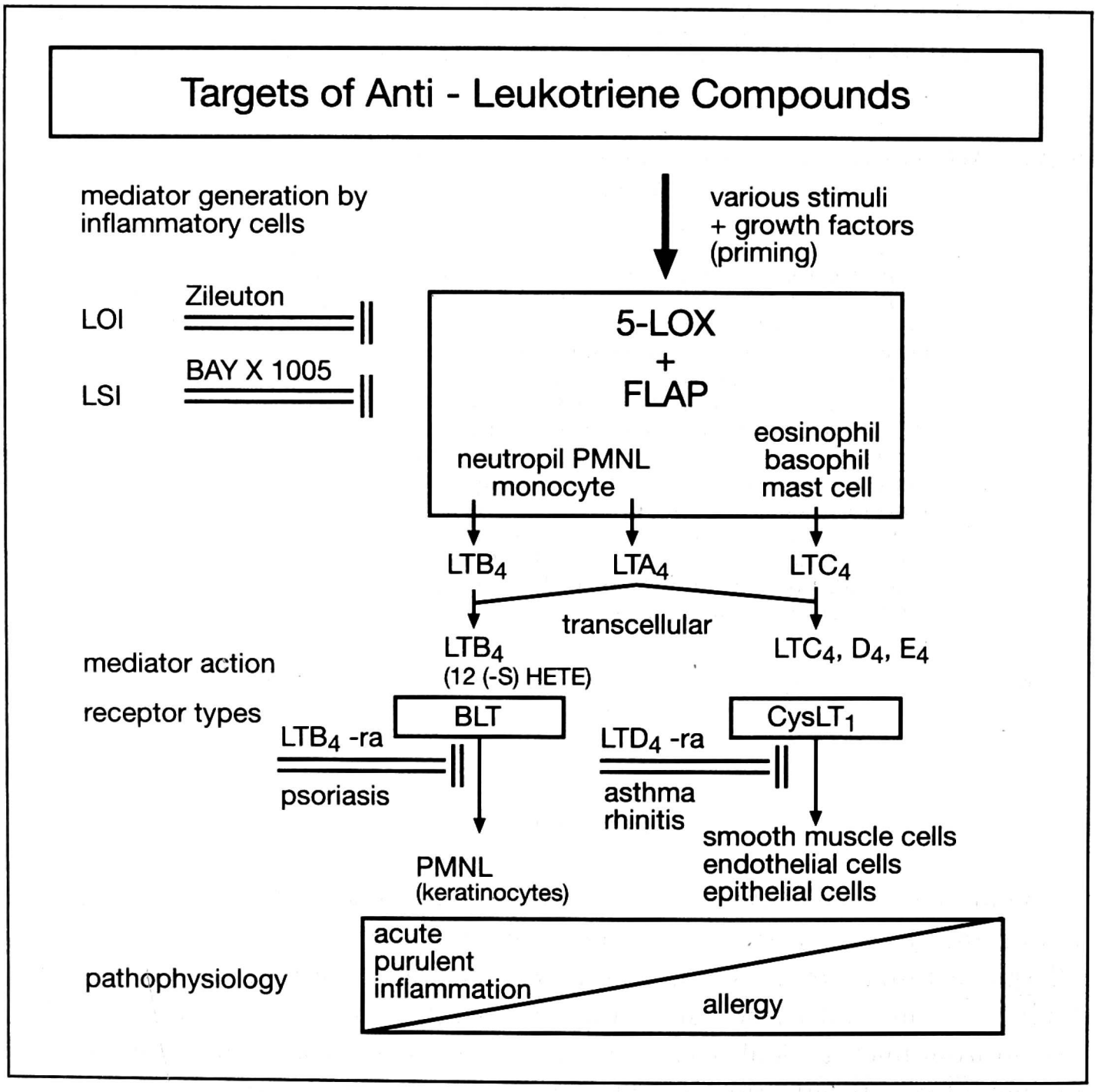

Fig. 1. Targets of anti-leukotriene compounds LOI: direct 5-lipoxygenase inhibitors; LSI: leukotriene synthesis inhibitors acting on the five lipoxygenase activating protein (FLAP); LTB_4 receptor antagonists acting on the BLT receptor; LTD_4 receptor antagonists acting on the $CysLT_1$ receptor.

metabolized by the synthetizing cell to LTB_4 or LTC_4, respectively or is transcellularly facilitated to neighbouring cells which according to their dominant enzymatic set-up metabolize LTA_4 to either LTB_4 or LTC_4. This fact might be of considerable pathophysiological and pharmacological importance as PMNL are throught to generate LTB_4 whereas considerable amounts are found to be metabolized to LTC_4 either by platelets or endothelial cells (6, 7).

Once the leukotrienes are generated their interaction with the respective receptor can be blocked by receptor antagonists. LTB_4 receptor antagonists prevent the interaction with the LTB_4 receptor, named BLT. LTB_4 has been found in numerous inflammatory conditions, but until now no clinical benefit could be demonstrated for LTB_4 receptor antagonists. This fact might be explained by the very redundant processes of acute inflammation where the blockade of a single pro-inflammatory mediator is of little therapeutic value because other leukotaxins than LTB_4 drive inflammation resulting in leukocyte infiltration.

LTC_4 interacts predominantly with the $CysLT_1$ receptor found in smooth muscle cells of arteries and bronchial airways. Various $CysLT_1$ receptor antagonists have been designed, first are now in use to treat symptomatic allergic asthma (8). $CysLT_2$ receptor antagonists are under experimental studies and thought to have the potential to treat pulmonary hypertension (9).

Leukotrienes found in different pathophysiologies

Various experimental and clinical inflammatory conditions have been analyzed and leukotrienes found. Yet, there is at present only one acute allergic inflammatory disease complex, which responds to anti-leukotriene compounds that is allergic asthma, including allergic rhinitis.

It is trivial, but has to be remembered that only the use of selective compounds will proove whether in complex-inflammatory conditions a certain mediator is of biological significance. First generation LOI exerted 5-LOX inhibition and overt anti-oxidative effects. The latter surely contributed to the overall anti-inflammatory effect and made researchers believe into the efficacy of 5-LOX inhibitors.

In vitro potencies

A number of conditions govern relative potencies of pharmacological agents. The LSI MK-886 ranks among the most potent 5-lipoxygenase inhibitors if one takes LTB_4 synthesis inhibition in human, rat or mouse PMNL as a measure ($IC50 \sim 0.09$ µmol/l) (10). The Schulz-Dale test which uses the guinea-pig tracheal contraction after antigen provocation *in vitro* ranks

MK-886 as weak (11). The higher potency of the LSI BAY X 1005 over MK-886 in the guinea-pig trachea test was reflected by studies on human lung tissue. Thus, tissue differences appeared more important than stimulus effects. BAY X 1005 was most potent in rat PMNL to inhibit LTB_4 synthesis (IC50 0.026 µmol/l) as compared to human PMNL (IC50 0.22 µmol/l). These potencies reflect BAY X 1005 binding to its target molecule FLAP (12).

Yet, the higher potency of BAY X 1005 in the rat does not translate into a high potency *in vivo*. BAY X 1005 and other anti-leukotriene compounds are virtually inactive in experimental inflammatory conditions of the rat where cyclooxygenase inhibitors are active (13). This seemingly contradictory effect is explained at least in part by a LTB_4 receptor defect of the rat (14).

These contradictions have to be reconciled when one decides for or against further compound development, or the use of 5-LOX inhibitors in so far new indications.

Anti-inflammatory effects of 5-lipoxygenase inhibition

The anti-inflammatory profile of anti-leukotriene compounds can only be estimated experimentally if they are sufficiently selective. First generation LOI exerted significant antioxidative properties besides 5-lipoxygenase inhibition. The LBI BAY X 1005 proved to be devoid of any antioxidant effects and inhibition of substrate generation *via* PLA_2, cyclooxygenase and 12-LOX inhibition (10, 12).

Anti-edematous effects

As already outlined, the rat carrageenin edema was found unresponsive to selective LSI and LOI (13). Thus, a newly developed model was used for this type of compounds i.e. the arachidonic acid-induced mouse ear inflammation test (AA-MEIT). BAY X 1005, topically tested, ranked among the most potent compounds (ED50 18 µg/ear; MK-886 38,5 µg/ear). It is of interest, that in this experimental model the efficacy of LOI and LSI is rather limited to about 60% (13). This indicates to other mediators being operative e.g. histamine and serotonine *via* H_1 and 5-HT receptors (15).

Even in this artificial experimental model could glucocorticosteroids add to the anti-edematous effects of BAY X 1005, even though the substrate for 5-LOX was directly applied to the mouse skin (Burchardt + Müller--Peddinghaus, manuscript under preparation).

Inhibition of PMNL adhesion

Leukotrienes promote leukocyte emigration from the microvascular site into the tissue. In addition to the leukotaxin LTB_4, LTC_4 is an important

activator of the microvascular endothelium to promote leukocyte adhesion, a prerequisite for leukocyte emigration (3, 6).

BAY X 1005 reduces PMNL adhesion after short-lasting (10 min) ischemia in the hamster cheek pouch model between 2.5 and 10.0 mg/kg p.o. (C. Gerdes, pers. comm.). No effects on exudate volume and cellularity could be seen in a rat pleurisy model further stressing the lack of response in this species. Yet, again in the arachidonic acid-induced mouse ear inflammation test (AA-MEIT) MPO level, reflecting PMNL influx at 4 hours, were significantly reduced (ED 50 7.9 mg/kg p.o.) (13).

Complex acute inflammation

A complex experimental model of inflammation: the zymosan-accelerated collagen-induced arthritis in the mouse responded to BAY X 1005. There was edema formation inhibition and reduction of acute arthritis of the zymosan-injected ankle joint and inhibition of joint destruction at dose of 25 mg/kg p.o. twice a day (17). These data clearly indicate to the potential of LSI to curb acute inflammation. The only 5-LOX-inhibitor tested in patients with rheumatoid arthritis was Zileuton, yet showing little benefit (18).

Allergic asthma represents another inflammatory disease with elevated CysLT generation as measured by enhanced urinary excretion of LTE_4 (19). Experimental studies focus on the functional lung parameters as related to the bronchospastic actions of CysLT, both *in vitro* and *in vivo* (11, 20, 21). Most studies were done using human and guinea-pig lung tissue or the breathing guinea-pig. Effects on the provoked infiltration of eosinophils was documentated even in Brown Norway rats (22).

Various clinical trials employing LOI, LSI and $CysLT_1$ receptor antagonists indicate significant symptomatic improvement of the lung function in allergic asthma patients. Yet, little effects are so far documented in the underlaying inflammatory process. Of interest is the favourable combination of anti-leukotriene therapy with moderate doses of glucocorticosteroids as shown experimentally and even clinically (Burchardt + Müller-Peddinghaus, manuscript under preparation (23). Other inflammatory diseases like psoriasis and inflammatory bowel disease so far failed to respond to treatment with 5-LOX-inhibitors (24, 25).

The finding of an enhanced excretion of LTE_4 in urine of patients with unstable angina indicated the presumed pathophysiological relationship of inflammatory vasculopathies with increased CysLT generation (26). Subsequent experimental studies indicate cardioprotective effects of BAY X 1005 in acute myocardial infarction in the rabbit (27). Yet, it is unclear whether this is the consequence of anti-inflammatory effects of this LSI or functional improvement achieved by other pharmacodynamic effects, yet to be identified.

Concluding remarks

The novel antileukotriene principles LOI, LSI and leukotriene receptor antagonists especially $CysLT_1$ receptor antagonists demonstrated anti--inflammatory effects in various animal models. Yet, only part of this promise translated into clinics. Whereas rheumatois arthritis, inflammatory bowel disease and psoriasis failed to respond to acute leukotriene therapy only symptoms of allergic asthma improved. This indicates the dominant role of CysLT in allergic asthma. One could speculate that other inflammatory diseases with a predominant role of CysLT might also respond to these novel therapeutic agents.

In the past, LTB_4 was probably overemphesized as pro-inflammatory mediator. The identification of the importance of the transcellular metabolism of LTC_4 from PMNL-generated LTA_4 tipped the balance even more towards a dominant role of CysLT in inflammation as exemplified by allergic asthma.

Thus, the potential of 5-LOX-inhibition to curb inflammation appears limited due to the redundant nature of inflammation. The potential risks of 5-lipoxygenase inhibition on the other hand appear to be neglible.

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