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Cysteinyl leukotriene-receptor-1 antagonists interfere with PGE_2 synthesis by inhibiting mPGES-1 activity

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ABSTRACT

Because of their favourable safety profile and beneficial anti-inflammatory properties, the $CysLT_1$ receptor antagonists (LTRA), montelukast, zafirlukast and pranlukast are approved for the treatment of asthma and are frequently prescribed as add-on therapeutics to reduce the amount of inhaled glucocorticoids and β_2-agonists. There is evidence that some of these anti-inflammatory properties might be of a secondary nature and therefore, unrelated to the $CysLT_1$ antagonism. Here, we show that LTRA inhibit PGE_2 formation in cytokine-stimulated Hela and A549 carcinoma cells and in lipopolysaccharide (LPS)-stimulated human leukocyte preparations ($IC_{50} \sim 20$ μM). Neither expression of enzymes involved in PGE_2 synthesis nor arachidonic acid release and COX activities were inhibited by the compounds. In contrast, mPGES-1 activity was suppressed at low micromolar levels (IC_{50} between 2 and 4 μM). This suppression was specific for PGE_2 synthesis, since PGD_2 and PGI_2 levels in LPS-stimulated leukocyte preparations were not negatively affected. $PGF_{2\alpha}$ levels were concomitantly inhibited, probably due to its direct synthesis from PGE_2. Several major conclusions can be drawn from this study: (A) clinical trials investigating elevated doses of the compounds are helpful to confirm suppression of PGE_2 synthesis *in vivo*; (B) studies investigating the role of CysLTs in cell culture or animal models of inflammation and cancer have to be reassessed carefully, if higher doses of LTRA were applied or serum levels in cell culture assays were low; and (C) LTRA may serve as new scaffolds for the development of potent, selective and well tolerated mPGES-1 inhibitors.

1. Introduction

Montelukast, zafirlukast and pranlukast are antagonists at the cysteinyl leukotriene receptor-1 ($CysLT_1$), a G protein-coupled receptor activated by the 5-lipoxygenase (5-LO) derived cysteinyl leukotrienes (CysLTs) LTC_4, LTD_4 and LTE_4. $CysLT_1$ is primarily located in the plasma membrane of inflammatory cells such as monocytes, macrophages, granulocytes, mast cells, lymphocytes and dendritic cells. In addition, epithelial cells, smooth muscle cells and fibroblasts express $CysLT_1$. CysLTs belong to the family of eicosanoids that are arachidonic acid (AA)-derived lipid mediators. Being among the most potent bronchoconstrictors yet studied in man, CysLTs play a crucial role in respiratory tract diseases such as asthma and allergic rhinitis (AR) [1]. In concert with LTB_4, histamine and PGE_2, CysLTs are thought to maintain the tone of human airways [2]. They promote maturation and migration of leukocytes from the

Abbreviations: AA, arachidonic acid; AR, allergic rhinitis; BMI, body mass index; COX, cyclooxygenase; $cPLA_{2\alpha}$, cytosolic phospholipase $A_{2\alpha}$; CSS, Churg-Strauss syndrome; CysLT, Cysteinyl leukotriene; DC, dendritic cell; FCS, fetal calf serum; GC, glucocorticoid; GI, gastro intestinal; IL-1β, interleukin-1β; 5-LO, 5-lipoxygenase; LC–MS/MS, liquid chromatography coupled with tandem mass spectrometry; LPS, bacterial lipopolysaccharide; LT, leukotriene; LTRA, Cysteinyl leukotriene receptor-1 antagonist; mPGES-1, microsomal prostaglandin E_2 synthase-1; NSAID, non-steroidal anti-inflammatory drug; PGE_2, prostaglandin E_2; PMNL, polymorphonuclear leukocytes; PBMC, peripheral blood mononuclear cells; TGFβ, tumour growth factor β; TNFα, tumour necrosis factor α.

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bone marrow and display chemoattractant properties on eosinophils, triggering cellular adhesion and transendothelial migration into the lung [3]. They also increase eosinophil survival in response to mast cell and lymphocyte paracrine signals and activate mast cells, eosinophils, T-lymphocytes, monocytes and basophils [4]. In addition, CysLTs seem to be involved in various other inflammatory conditions such as cancer, cardiovascular, gastrointestinal, skin and immune disorders [1]. Therefore, inhibition of the LT pathway is a promising approach for the development of drugs for the treatment of atopic diseases. So far, four compounds have been approved. These are the 5-LO inhibitor zileuton, and the $CysLT_1$ receptor antagonists (LTRA) montelukast, zafirlukast and pranlukast (Fig. 1). The use of zileuton, however, is limited by its short half-life (application four times daily, 600 mg) and elevation of hepatic enzymes. Therefore, LTRA are the most frequently used LT modifiers. Antagonizing effectively the pro-asthmatic and pro-inflammatory activities of CysLTs, these compounds are primarily used as add-on therapeutics to reduce the amount of inhaled glucocorticoids (GC) and β_2-agonists in the management of asthma and AR. Being very well tolerated, LTRA show good efficacy in the inhibition of short-term bronchoconstriction, potently inhibit bronchial inflammation and exert strong GC-sparing effects [5–8]. There is some evidence that aspects of these anti-inflammatory properties might be of a secondary nature and therefore, unrelated to the $CysLT_1$ antagonism. Recently, Tintinger et al. summarized such properties for montelukast [9], including the inhibition of 5-LO [10], of TNFα-dependent IL-8 expression via interference with NFκB associated histone acetyltransferase activity [11], cAMP phosphodiesterase inhibition [12] and inhibition of adhesion and migration of eosinophils in the vasculature [13]. Here, we report that LTRA inhibit PGE_2 formation in cytokine-stimulated cervix and lung carcinoma cell lines, as well as LPS-stimulated leukocyte preparations from human whole blood by suppression of microsomal prostaglandin E_2 synthase-1 (mPGES-1) activity.

2. Materials and methods

2.1. Cell culture

The human cervix carcinoma cell line Hela, as well as A549 lung cancer cells were purchased from Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). All cell lines were maintained in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% fetal calf serum (FCS), 1 mM sodium pyruvate, 100 μg mL^{-1} streptomycin and 100 U mL^{-1} penicillin. Cells were kept at 37 °C, in a humidified atmosphere containing 5% CO_2.

2.2. Determination of prostaglandin concentrations in cell culture supernatants

A549 or Hela cells (0.25×10^6, 0.15×10^6 cells per well, respectively) were seeded in normal growth medium into 6-well plates and allowed to attach for 24 h at 37 °C, 5% CO_2. The incubation was started by replacing medium by 2 mL normal growth medium (Hela) or growth medium with 2% FCS (A549). In addition, IL-1β, 1 ng $mL^{-1}$and TNFα 5 ng mL^{-1} (Hela) or IL-1β, 1 ng mL^{-1} alone (A549) were added in presence of the inhibitors. Cells were incubated for 16 or 24 h (Hela and A549, respectively). For experiments on serum dependency, increasing concentrations (5, 10, 20%) of FCS were added and cells were incubated for 4 h. After this, media were collected, placed on ice and centrifuged (2000 rpm, 5 min, 4 °C) to remove residual cells. PGE_2 concentrations were analyzed via LC–MS/MS.using an API 5500 triple quadrupole mass spectrometer (Applied Biosystems, Darmstadt, Germany) as described previously [14]. All experiments were performed in triplicate.

2.3. Human whole blood assay

Aliquots of heparinized human whole blood (500 μL) were incubated with LPS (10 μg mL^{-1}) plus $CysLT_1$ receptor antagonists

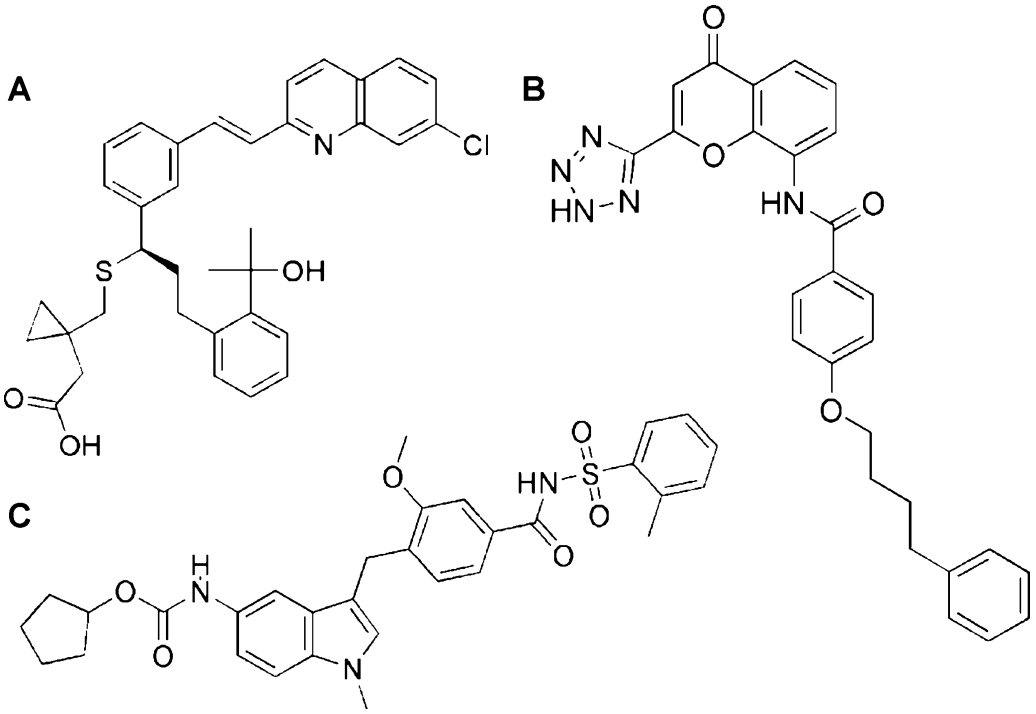

Fig. 1. Chemical structures of the three approved LTRA montelukast (A), pranlukast (B), zafirlukast (C).