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Institutionen for Medicin Huddinge, Enheten för Hjärt- och Lungsjukdomar

Clinical studies on the role of eicosanoids in the asthmatic airway inflammation

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ABSTRACT

The underlying mechanisms in the asthmatic airway inflammation involve the interaction between different inflammatory cells and mediators that consequently result in different clinical phenotypes. The aim of this thesis was to investigate the impact of inflammatory mediators, with emphasis on eicosanoids, on the inflammatory and functional airway responses under basal and triggered conditions in subjects with asthma, in particular ASA/NSAID-intolerant and allergic phenotypes. In the studies included in this thesis, we investigated the possibility of finding new phenotype-specific biomarkers of asthma in connection with mechanistic pathways of eicosanoid biosynthesis.

Eleven aspirin-sensitive asthmatics had, in comparison with ten aspirin-tolerant asthmatics, higher exhaled nitric oxide levels and higher baseline levels of CysLTs in saliva, sputum, blood ex vivo and urine. Levels of urinary LTE_4 and 9α,11β-prostaglandin F_2 increased after aspirin provocation whereas leukotriene levels in saliva and ex vivo stimulated blood did not increase. These findings support a selective CysLT-overproduction in this distinct clinical syndrome. CysLTs in saliva should be explored as a new and clinically convenient biomarker of AIA and other diseases associated with increased production of leukotrienes.

In an explorative study, the capacity of eosinophils to produce 15-LO pathway products and their *ex vivo* responsiveness to COX inhibition was studied in the peripheral blood drawn from healthy volunteers and three asthma groups. In the absence or presence of lysine-aspirin, eosinophils were stimulated with arachidonic acid and calcium ionophore to trigger the 15-lipoxygenase-1 (15-LO) and 5-lipoxygenase (5-LO) pathways, respectively. The results displayed an increased release of the recently discovered lipid mediator eoxin C_4 (EXC_4) as well as the main indicator of 15-LO activity, 15-HETE, in activated eosinophils from severe and aspirin-intolerant asthmatics. Eosinophils from AIA subjects also showed elevated EXC_4 and LTC_4 formation after cellular activation in the presence of lysine-aspirin. This higher biosynthetic activity of 15-LO pathway in AIA is in part due to increased numbers of eosinophils, but the data also support enhanced eosinophil function, possibly involving transcellular interactions with platelets. The findings support contribution of 15-LO pathway in the pathophysiology of severe and aspirin-intolerant asthma.

This thesis also aimed at evaluating the role of COX-1 and COX-2 in the biosynthesis of the pro-inflammatory prostaglandin D_2 (PGD_2) and bronchoprotective prostaglandin E_2 (PGE_2) under basal conditions and during heightened airway inflammation and responses after inhaled allergen provocation. Eighteen subjects with asthma and six healthy controls participated in a cross-over study where a selective COX2 inhhibitor, celecoxib 200 mg, or placebo were given b.i.d. on 3 consecutive days following 2 untreated baseline days. Celecoxib treatment inhibited urinary excretion of the tetranor metabolite of PGE_2, PGEM, by 50% or more in asthmatic subjects and healthy controls, whereas there was no significant change in the excretion of the tetranor metabolite of PGD_2, PGDM. In addition, celecoxib did not cause any significant changes in FEV1 or FENO. In comparison with the healthy controls, the subjects with asthma had higher baseline levels of urinary PGDM but not of PGEM. These findings indicate that biosynthesis of PGD_2 is catalysed predominantly by COX-1 and that COX-2 contributes substantially to the biosynthesis of PGE_2. The asymmetric impact of COX-2 inhibition on prostanoid formation raises the possibility of long-term adverse consequences of COX-2 inhibition on airway homeostasis by the decreased formation of PGE_2 and maintained production of increased levels of PGD_2 in asthmatics.

Therefore, the effect of selective COX-2 inhibition on induced asthmatic airway obstruction and inflammation was investigated in 16 subjects with mild atopic asthma who underwent with rising dose inhalation challenges with allergen and methacholine (MCh) to determine the provocative dose causing a 20% drop in FEV_1 (PD_{20}) during a control study period and following 10-13 days of treatment with etoricoxib (90 mg once daily). Study periods were randomized with at least 2 weeks washout between and induced sputum cells and exhaled nitric oxide levels (F_ENO) were used to assess airway inflammation. Blood assays for COX-1 and COX-2 activity to determine biochemical efficacy were performed and urinary excretion of lipid mediators was measured by mass-spectrometry. The intervention with COX-2 inhibitor in provoked asthma was not found to have any negative effects on allergen-induced airflow obstruction and sputum eosinophils, basal lung function or methacholine responsiveness. The study suggests that short-term use of COX-2 inhibitors is safe in asthmatics.

In summary: 1) The higher baseline LTE_4-levels found in three body matrices lends further support to CysLT-overproduction in AIA and the higher salivary levels should be explored as a new and clinically convenient biomarker of AIA and other diseases with increased CysLT-production. 2) The increased release of the 15-LO products, EXC_4, and 15-HETE, in activated eosinophils from severe asthma and AIA patients, and the elevated EXC_4 and LTC_4 formation in activated eosinophils from AIA subjects in the presence of ASA support a pathophysiological role of the 15-LO pathway in AIA and severe asthma. 3) Basal biosynthesis of PGD_2 is increased in subjects with asthma and its formation is catalysed predominantly by COX-1. By contrast, COX-2 contributes substantially to the biosynthesis of PGE2. 4) COX-2 inhibition in provoked asthma is found to have no negative effects on allergen-induced airflow obstruction and sputum eosinophils, basal lung function or MCh responsiveness suggesting that short-term use of COX-2 inhibitors is safe in asthmatics.