Regulatory mechanisms of leukotriene synthesis and degradation in childhood bronchial asthma

A brief report from the 5th Asia Pacific Congress of Allergology and Clinical Immunology, Seoul (Korea) Oct 12-15, 2002

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Key words: bronchial asthma, cysteinyl leukotriene (cysLT), LTB4, 5-lipoxygenase, LTC4 synthase

Summary

Cysteinyl leukotrienes (cysLTs), arachidonate 5-lipoxygenase products, play significant roles in the pathogenesis of bronchial asthma in children. In these 15 years several lines of evidence have demonstrated that the production of cysLTs is controlled by molecular mechanisms induced by external and internal bioactive substances. A direct evidence is that levels of cysLTs and LTB4 in BALF were markedly increased in asthmatic children with acute exacerbation. Peripheral polymorphonuclear leukocytes obtained from asthmatic children produced higher levels of cysLTs and LTB4 than those from controls, which also supported this hypothesis. The increased production of cysLTs and LTB4 is attributable to transcriptional and/or post-transcriptional up-regulation of LT-synthetic enzymes, 5-lipoxygenase, LTA4 hydrolase and LTC4 synthase. Activity of cysLT-catabolic enzymes in vivo is also determined by degradation of cysLTs. LTC4 is metabolized to less bioactive LTE4 via LTD4 by two consecutive catabolic enzymes, gamma-glutamyl leukotriene(gamma-glutamyl transpeptidase related enzyme; GTPRE) and dipeptidase. GTPRE was found to be transcriptionally up-regulated by glucocorticosteroid(GCS) in transformed human bronchial epithelial cells. Accelerated conversion of LTC4 to LTE4 by GCS-induced GTPRE explains, in part, the efficacy of GCS in the treatment of bronchial asthma. Thus, leukotriene metabolism is controlled by transcriptional and/or post-transcriptional regulatory mechanisms at various steps of synthetic and catabolic enzymes, which contributes to pathophysiology of childhood asthma.

Introduction

Cysteinyl leukotrienes (cysLTs; a mixture of LTC4, LTD4 and LTE4), potent bioactive substances, are synthesized from arachidonic acid by a series of enzymatic reactions in mast cells, monocytes, eosinophils and basophils in response to allergic and other inflammatory stimuli \(^1 \text{ - } ^3\). CysLTs, especially LTC4 and LTD4, produce a sustained contraction of airway smooth muscle, hyper-secretion of bronchial glands, and an increased permeability of the vascular...
Inhaled authentic LTD4 resulted in increasing eosinophil migration into the bronchial epithelium and subepithelial areas in human, which was inhibited by 5-lipoxygenase inhibitor. CysLTs inhibited the apoptosis of eosinophils that were important effector cells in allergic airway inflammation. CysLTs potentiated EGF-induced proliferation of human airway smooth muscle cells in vitro. More recently, it was reported that cysteine receptor antagonist inhibited allergen-induced chronic lung inflammation (deposition of collagen beneath the airway epithelial cell layer, goblet cell hyperplasia) in a mouse model. These results indicate the involvement of cysteine LT not only in acute allergic reaction but also in possible chronic airway inflammation and the following airway remodeling that is characterized by bronchial asthma.

Production of cysteine LTs is initiated by the liberation of arachidonic acid (AA) from membrane phospholipid by an activation of AA-specific phospholipase A2 (PLA2) in the cytosolic fraction of the cells. The liberated AA is enzymatically converted to 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and then to unstable epoxide LTA4 by 5-lipoxygenase (5-LO) which is translocated from cytosol to nuclear membrane. The action of 5-LO is associated with 5-LO activating protein (FLAP). This unstable LTA4 epoxide is enzymatically converted to LTC4 by LTC4 synthase. LTC4 is then converted to LTD4 by GTPRE and then further metabolized to LTE4 by cysteinylglycine dipeptidase. Recent in vitro studies have demonstrated that these enzymes and the protein molecule are transcriptionally and/or post-transcriptionally up-regulated by various bioactive substances such as various interleukins (ILs), growth factors and exogenous pharmacological substances such as glucocorticosteroid. These findings suggest that these enzyme-activities are controlled by such molecular mechanisms and are possibly up-regulated in bronchial asthma.

The production of cysteine LTs in BALF is increased in childhood asthma

Concentrations of cysteine LTs expressed as LTC4, and of LTB4 in bronchoalveolar lavage fluid (BALF) were measured by specific RIA in childhood asthma patients. The concentrations of LTC4 and LTB4 in BALF were apparently increased in asthma exacerbation. Before extubation, the levels of LTB4 and LTC4 were increased.
measured again when asthma symptoms subsided. The concentrations of both LTB4 and LTC4 were under minimal detectable levels. Thromboxane(TX) B2 could not be detected in acute and convalescent phases in these patients. These results indicate that cysLTs and LTB4, but not TXA2, are synthesized in great quantities in the airways and play important roles in the pathogenesis of acute asthma exacerbation in children16).

The productivity of cysLTs in peripheral PMN in asthmatic patients is enhanced:

We demonstrated that PMNs obtained from asthmatic children without attacks produced significantly greater amounts of LTB4 and LTC4 than those from control subjects. In order to determine the mechanisms why the production of LTs was increased in asthmatic patients, we examined mRNA expression of a series of synthetic enzymes. Expression of mRNA of 5-lipoxygenase, LTA4 hydrolase and LTC4 synthase measured by RT-PCR was enhanced in asthmatic children without attacks. Surprisingly, however, the enhanced productivity of cysLTs and LTB4 was returned to the control level in acute asthma exacerbation. The reason why LT-synthesis was returned to the control levels in asthma attack was not clear. A possible explanation is that the activated inflammatory eosinophils and neutrophils which have higher enzymatic activities may be recruited to local inflammatory sites in acute asthma exacerbation16. These results suggest that there are some regulatory mechanisms which transcriptionally up-regulate the activities of LT-synthetic enzymes in PMNs in asthmatic patients. 17.

Conversion rate of LTC4 to LTE4 in asthmatic children:

Catabolism of LTC4 to less bioactive LTE4 is another important aspect in determining the action of cysLTs. We examined enzyme-activity in sera which converts LTC4 to LTE4 in the patients with asthma and compared it with that in control subjects. Externally added authentic LTC4 was incubated for 30 min with sera obtained from patients or control subjects, and the levels of LTC4 and LTE4 were measured by HPLC. In the patients with asthma, the level of LTC4 was higher than that in the controls, and the level of LTE4 was lower than that of the controls, indicating that the conversion of LTC4 to LTE4 was decreased in asthmatic patients18.

Action of GCS in catabolic pathway of cysLTs:

Catabolic pathway of LTC4 to LTE4 is an important aspect to consider asthma treatment, because LTE4 is biologically less active than LTC4 and LTD4. LTC4 is converted to LTD4 by GTPRE, and then further metabolized to LTE4 by dipeptidase. (fig 2) We examined whether glucocorticosteroid(GCS) and other anti-asthma drugs have any pharmacological effects on the activities of these catabolic enzymes in transformed human bronchoepithelial cells (16HBE cells). 16HBE cells were incubated with or without GCS for 2 days, then authentic LTC4 was added into the medium. Conversion of LTC4 to LTE4 via LTD4 was measured by HPLC. GCS treatment increases LTC4 catabolic activity to LTE4. The same activity was examined in the presence of salbutamol and DSCG. Salbutamol and DSCG did not increase LTC4 catabolism. Only GCS accelerated the conversion of externally added LTC4 to LTD4 and LTE4. In order to clarify the molecular mechanisms of this acceleration, mRNA expression of catabolic enzymes, gamma glutamyl transeptidase and GTPRE was examined by RT-PCR. Messenger RNA expression of GTPRE was enhanced, indicating that GCS attenuated bioactivities of cysteinyl LTs by transcriptionally up-regulating GTPRE, a catabolic enzyme19).

Discussion and conclusion

We demonstrated that production of cysLTs and LTB4 in the airway was increased in acute asthma, and the productivity of cysLTs in peripheral PMNs was enhanced by molecular mechanisms of LT-synthetic enzymes, 5-LO, LTA4 hydrolase and LTC4 synthase. Pouliot et al demonstrated that granulocyte-macrophage-
stimulating factor (GM-CSF) induced dose- and time-dependent de novo synthesis of 5-lipoxygenase in PMNs(12). Western blot analysis showed that GM-CSF induced a rapid increase in the total cellular level of 5-lipoxygenase protein, indicating a translational effect of GM-CSF on the expression of the 5-lipoxygenase. On the other hand, Stankova et al demonstrated that transcriptional rate of 5-lipoxygenase mRNA was increased without affecting the degradation ratio in human PMNs after 18 h incubation with GM-CSF, indicating that LT synthesis was transcriptionally regulated at 5-lipoxygenase gene level20. Other investigators reported that GM-CSF and TNFα up-regulated mRNA expression and protein synthesis of FLAP in human PMNs21. Thus, LT synthesis appears to be dually regulated at 5-lipoxygenase and FLAP levels.

Regarding LTC4 synthase, Hsieh et al demonstrated that IL-4 induced the expression of LTC4 synthase mRNA and the protein in SCF-primed human mast cells derived from cord blood mononuclear cells14. We also found that retinoic acid (RA) potently induced LTC4 synthase activity and resulted in markedly increased production of cysLTs in rat basophilic leukemia-1(RBL-1) cells22–24. We examined the effect of dexamethasone (DEX) on RA-induced LTC4 synthase activity. The induction of LTC4 synthase was effectively suppressed by DEX. Northern blotting analysis, however, showed that the expression of mRNA for LTC4 synthase was not affected by the exposure to RA or DEX, indicating that RA-induced enhancement of LTC4 production and its inhibition by DEX was determined by post-transcriptional regulation of LTC4 synthase24. Thus, LTC4 synthase is confirmed to be up-regulated by transcriptional and/or post-transcriptional mechanisms.

In previous reports, investigators reported that LT-production in PMNs was increased in patients with bronchial asthma,25, 26 but the mechanisms; especially possible molecular mechanisms of LT-synthetic enzymes, had not been clarified. In our studies, we have demonstrated that increased LT production in asthma patients is attributable to up-regulation of synthetic enzymes, 5-LO, LTA4 hydrolase and LTC4 synthase. We also found that catabolic pathway of LTC4 to LTD4 was accelerated by glucocorticosteroids by new induction of GTPRE in transformed human bronchoepithelial cells, which finally lead to the accelerated degradation LTC4 to less active LTE4 via LTD4. This is a novel regulatory mechanism by glucocorticosteroids on LT metabolic pathway. Thus, molecular mechanisms regulating LT-synthesis have been confirmed in diverse cell types and various conditions. (fig 3) We have to further investigate more precise mechanisms how cysLTs and LTB4 participate in the pathogenesis of childhood asthma.
regulatory mechanisms of LT metabolism

Figure 3. Various bioactive substances activate or inactivate LT-synthetic and catabolic enzymes

References


13) Cowburn AS, et al. IL-5 increases expression of 5-lipoxygenase-activating protein and translocates 5-lipoxygenase to the nucleus in human blood


和文抄録

システィニルロイコトリエン（cysLTs）はアラキドン酸の5リボキサンギナーゼ代謝物で、小児気管支喘息の病態の発現に重要な役割を果たしている。cysLTsの合成は外的、内的な活性物質により分子生物学的機序で制御されていることがこの15年間の研究で明らかになってきた。CysLTsが小児の気管支喘息で重要な役割を果たしているという直感的な証拠は、患児の大発作時に採取した肺洗浄液中にcysLTsとLTB4の濃度が著しく高値であるというところ、喘息患者の末梢血中性白血球のcysLTsおよびLTB4産生能が、コントロール児よりも有意に高いことによって示される。このcysLTsおよびLTB4産生能の上昇は、合成酵素である5リボキサンギナーゼ、LTC4合成酵素、LTA4脱水素酵素の転写もしくは翻訳の活性上昇に由来することが明らかになった。CysLTsの生物学的活性はその分解の速度によっても影響を受ける。LTC4は連続的に作用する2つの酵素、ガンマグルタルミロイコトリナーゼ（GTPRE）とジェチチダーゼ、によってLTD4、LTE4へと連続的に変換される。LTE4は生物学的活性が低いので、この分解酵素系が活性化されるとcysLTsの活性が影響を受ける。ヒトの培養気道上皮細胞において、GTPREはステロイドにより転写が活性化され、酵素活性が上昇することが明らかとなった。ステロイドによるGTPRE誘導の機序は、吸収ステロイドが喘息に有効である理由のひとつであると考えられる。このように、ロイコトリエンの代謝に関与した多くの酵素は分子生物学的な制御を受けており、この制御は喘息の病態に深く関与していると考えられる。