LEADING ARTICLE

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The Potential Role of Tocopherol in Asthma and Allergies

Modification of the Leukotriene Pathway

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Abstract

Metabolism of arachidonic acid via the 5-lipoxygenase (5-LO) pathway leads to the formation of hydroperoxyeicosatetraenoic acids (HPETEs) and leukotriene (LT) A_4. This unstable allylic epoxide can be further converted by secondary enzymes into LTB_4 and cysteinyl LTs. LTs represent a family of potent biologically active compounds synthesised by specific cell types and by transcellular biosynthetic mechanisms. Cysteinyl LTs are involved in the pathogenesis of asthma, and recent data indicate that individuals with asthma may have enhanced basal excretion of urinary LTE_4 compared with normal individuals.

Tocopherol (vitamin E) and tocopherol acetate strongly inhibit potato 5-LO in an irreversible and noncompetitive way, and, by affecting the redox state of cells possessing 5-LO, they may influence the production of biologically active LTs. It has been reported that normal plasma levels of tocopherol may enhance the lipoxygenation of arachidonic acid, whereas higher tocopherol levels exert a suppressive effect that is consistent with its role as a hydroperoxide scavenger.

Receptor-mediated activation of neutrophils in individuals with asthma results in the synthesis of LTs. This activation is inhibited by tocopherol in a concentration-dependent manner. Additional controlled studies are needed to assess the effect of tocopherol on leukotriene production in asthmatic individuals. The results of these studies may be useful in developing new therapeutic approaches in asthmatic/allergic patients.

1. The Leukotriene Pathway

Leukotrienes (LTs) are metabolites arising from arachidonic acid through the action of the enzyme 5-lipoxygenase (5-LO). The synthesis of LTs in cells possessing the appropriate enzymatic machinery can be triggered by a variety of soluble and particulate stimuli, including antigens, microbes, cytokines, immune complexes and model agonists such as calcium ionophores.

Phospholipase A_2 (PLA_2) initiates LT synthesis by catalysing the hydrolysis of arachidonic acid from membrane phospholipids.[1] The arachidonic acid released from cellular phospholipids is enzymatically oxygenated by 2 major pathways: cyclooxygenase enzymes, which convert arachidonic

acid into labile endoperoxides that are transformed to prostaglandins, thromboxane (TXA_2) and prostacyclin (PGI_2),[2] and lipoxygenase enzymes,[3,4] as well as into the biologically active LTs. The lipoxygenase enzymes metabolise arachidonic acid to unstable hydroperoxyeicosatetraenoic acids (HPETEs) that are transformed into monohydroxyeicosatetraenoic acids (HETEs).

The first committed enzyme in the LT synthetic pathway is represented by 5-LO, mainly found in cells of the leucocyte and monocyte lineage. This enzyme requires Ca^{2+} and ATP, and its efficient utilisation of endogenously released arachidonic acid in intact cells also requires an 18kD protein, named 5-LO activating protein (FLAP).[5] 5-LO catalyses the conversion of arachidonic acid into the unstable allylic epoxide LTA_4, which represents the precursor for all the bioactive LTs. It can be hydrolysed by LTA4 hydrolase to LTB_4, which has potent chemotactic and leucocyte-activating effects, or conjugated with reduced glutathione by LTC_4 synthase to yield LTC_4.

LTC_4 can be further modified extracellularly by sequential amino acid removal to yield LTD_4 and LTE_4, collectively known as the cysteinyl LTs (cys-LTs). These represent the smooth muscle contractile and vascular permeability activities long recognised as slow-reacting substance involved in anaphylaxis.[6] It was soon documented that in particular LTC_4 and LTD_4 were potent inducers of bronchoconstriction in guinea-pig airways *in vitro* and *in vivo*[7,8] and caused contraction of isolated human bronchi.[9-11]

2. Role of Leukotrienes in Asthma and Allergies

LTB_4 was the first LT discovered[12] during studies on the metabolism of arachidonic acid in rabbit leucocytes.[13] With the exception of a contractile effect in the guinea-pig lung parenchyma,[14] mainly resulting from secondary production of thromboxane A_2, inflammatory cells are the principal targets for the biological activity of LTB_4.

LTB_4 is a potent stimulus for activation of leucocytes, eliciting chemokinetic and chemotactic responses *in vitro*.[15] *In vivo*, LTB_4 increases leucocyte rolling and adhesion to the venular endothelium. This initial chemotactic response is followed by migration of leucocytes into the extravascular space.[16]

In addition to effects on leucocyte recruitment, LTB_4 stimulates secretion of superoxide anion and release of granular constituents from leucocytes.[17,18] Among the effects of LTB_4 on inflammatory cells, it may affect the expression of low affinity receptors for immunoglobulin E (IgE) on B-lymphocyte cell lines[19] and synthesis of IgE induced by interleukin-4.[20]

Concerning the biological activities of cys-LTs,[6] they are among the most powerful bronchoconstricting agents ever tested in humans. *In vivo*, cys-LTs cause bronchoconstriction both in healthy individuals and in patients with asthma[21] and they are considered to play a major role in aspirin (acetylsalicylic acid)-induced asthma.[22]

Elevated levels of immunoreactive LTC_4/LTD_4 have been reported in nasal lavage fluid in patients with perennial allergic rhinitis[23] and seasonal allergic rhinitis.[24] Elevated levels of LTs are also reported in nasal polyp tissue,[25] a condition associated with tissue eosinophil recruitment. The temporal release of LTs after allergen exposure has been explored using nasal allergen challenge and nasal lavage techniques[26-28] and their production correlates well with vascular modification resulting in oedema and nasal obstruction.

The role and physiological relevance of cys-LTs in the control of airways tone and resistance *in vivo* have been demonstrated in a series of clinical studies,[27,28] and leukotriene synthesis inhibitors, as well as LT receptor antagonists, are now widely used in the treatment of asthma.[29,30]

3. The Role of Oxidative Damage in Asthma

Asthma has been recognised as a chronic inflammatory disorder of the airways, involving inflammatory cell recruitment and activation. Infiltration and activation of phagocytes may result in significant amounts of reactive oxygen species

(ROS) being formed, and it has been reported that alveolar macrophages,[31] as well as eosinophils,[32] neutrophils and monocytes[33] obtained from individuals with asthma produce more ROS than cells obtained from normal individuals.

Recently, increased amounts of 8-isoprostane, a prostaglandin F_2-like compound produced by the free radical-catalysed peroxidation of arachidonic acid, have been detected in exhaled air from patients with asthma,[34] suggesting that these patients undergo significant oxidative stress *in vivo*.

In addition to increased oxidative stress, several findings reported in the literature suggest that individuals with asthma present with significantly impaired antioxidant potential: recently, a study of tocopherol (vitamin E) levels in bronchial wash and bronchoalveolar lavage from patients with mild asthma showed significantly lower levels than those observed in controls, while higher levels of oxidised glutathione were found.[35]

In 1986, Malmgren reported lowered whole blood glutathione peroxidase (GSH-Px) activity in patients with acetylsalicylic acid-induced asthma and food intolerance.[36] Subsequent observations in adults with asthma suggested reduced whole blood and plasma selenium content and reduced activity of GSH-Px compared with controls.[37-39]

Reduced protection from oxidative damage caused by free radicals and hydroperoxides released during the inflammatory process may contribute to worsening airways inflammation.[40,41] However, from current knowledge, it is unclear whether the low antioxidant status is a primary event associated with the pathogenesis of asthma or a secondary event related to the inflammatory process taking place in asthma, in which the high demand for antioxidant is outstripping the supply, thus exacerbating the free radical damage to the cellular environment.

ROS formation may lead to lipid membrane damage, as well as damage to other cell constituents such as DNA. Among the products formed by ROS, hydroperoxides arise from oxygen attack on unsaturated fatty acids esterified into membrane phospholipids. Together with a potential role in propagating radical-driven oxidative reactions, hydroperoxides may also play an important role in modulating the activity of various important enzymes. The presence of hydroperoxides is in fact necessary for the activity of both cyclo-oxygenase and 5-LO,[42] and modulation of 'hydroperoxide tone' may result in enhancement, as well as suppression, of 5-LO activity and LT production.

4. Potential Role of Tocopherol in Asthma and Allergies

On the basis of the abovementioned findings, antioxidants such as tocopherol, which protect the membrane phospholipids from the action of ROS and prevent peroxidation of polyunsaturated fatty acid including arachidonic acid,[43-45] may control the formation of arachidonic acid metabolites, and specifically of LTs.

In 1980, Goetzl reported that tocopherol bidirectionally modulates the activity of the lipoxygenase pathway of human neutrophils *in vitro*. Normal plasma levels of tocopherol enhance the lipoxygenation of arachidonic acid, as well as neutrophil chemotaxis, whereas higher levels (0.25 to 1 mmol/L) exert a suppressive effect both on chemotaxis and on the formation of lipoxygenase products, which is consistent with its role as hydroperoxide scavenger.[46]

In rat or human neutrophils, tocopherol deficiency results in greater than normal release of hydrogen peroxide, augmented peroxidation of membrane lipids and impairment of chemotaxis and phagocytosis. These events are corrected rapidly by addition of tocopherol *in vitro* or prevented by prior administration of several doses of tocopherol to the neutrophil donors.[47]

Exposure of neutrophils to tocopherol concentrations of 0.25 to 1 mmol/L increased the content of tocopherol in platelets and leucocytes by up to 5- to 17-fold within 5 minutes of incubation *in vitro*. A similar effect can be obtained *in vivo* after pharmacological doses of tocopherol.[48,49]

Villa and co-workers found that tocopherol *in vitro* inhibited not only arachidonate-induced, but also LTB_4-induced, aggregation of human neutro-

phils, suggesting that the effects of tocopherol on human leucocyte aggregation may reflect non-lipoxygenase-related activities.[50]

In vitro studies with isolated enzymes have suggested that cellular tocopherol levels may have a profound effect on the activity of 5-LO. These effects are at least partially based on nonreversible inhibition of the 5-LO oxidase function.[51]

Recently, we studied the effect of tocopherol supplementation *in vitro* on the formation of LTB_4 and its ω-oxidised metabolites in neutrophils obtained from individuals with asthma. Production of LTs was induced by activation with the formylated tripeptide N-formyl-methionyl-leucyl-phenylanine (fMLP), in the presence of exogenous arachidonic acid, in order to avoid the potential inhibitory effect of tocopherol on calcium-independent PLA_2.[52] The results obtained showed a significant inhibition of LT production at concentrations of 100 to 300 μmol/L (fig. 1), suggesting that tocopherol supplementation may indeed affect leukotriene formation in individuals with asthma.[53]

Limited data are available concerning the effect of tocopherol supplementation, asthma, and the formation of LTs *in vivo*.

Luostarinen and co-workers found that supplementation with a moderate dose of tocopherol in healthy volunteers increased the serum tocopherol level and significantly decreased neutrophil chemotaxis, but did not influence generation of LTB_4.[54] It must be underlined that these authors used the calcium ionophore A23187 at a concentration that appears unlikely to be modulated by changes in the hydroperoxide content resulting from tocopherol supplementation; furthermore, they evaluated LTB_4 by immunoassay, whereas in isolated neutrophils LTB_4 accounts only for a minor part of the 5-LO metabolites produced.[55]

In vivo leukotriene production was studied in individuals with a genetic tocopherol deficiency,[56] evaluating the urinary excretion of LTE_4; in these patients supplementation with tocopherol is promptly reflected in their plasma tocopherol concentrations. The results obtained showed an inverse correlation between urinary LTE_4 excretion and plasma tocopherol concentrations. Recent data demonstrated that in asthmatic patients, urinary LTE_4 may have increased basal excretion compared with non-asthmatic individuals.[57]

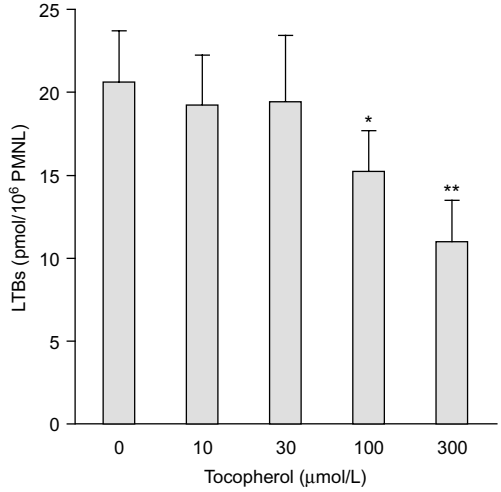

Fig. 1. Effect of tocopherol on the production of leukotriene B_4 and its ω-oxidised metabolites (LTB)s by neutrophils obtained from individuals with asthma. Peripheral blood neutrophils were purified by dextran sedimentation and centrifugation on a discontinuous Ficoll gradient. Neutrophils were activated with N-formyl-methionyl-leucyl-phenylanine [0.1 μmol/L] in the presence of arachidonic acid (5 μmol/L) for 10 minutes at 37°C. Metabolites were analysed by reverse-phase high performance liquid chromatography coupled to a diode-array UV detector and identified according to the retention time of synthetic standards and on-line UV absorbance spectrum analysis. Quantification was performed on positively identified peaks only. **PMNL** = polymorphonuclear leucocytes. * $p<0.05$; ** $p<0.01$ *vs* control; paired data analysis.

5. Conclusions

The results obtained by various groups of investigators indicate that LT production *in vivo* and *in vitro* may be modulated by tocopherol.

Most studies showed the effect of tocopherol, *in vivo* and *in vitro*, on the production of LTs and lipoxygenation of arachidonic acid in normal individuals. Limited data are available in asthmatic and allergic individuals. Our results provide support for the hypothesis of a potential relationship between tocopherol levels and 5-LO activity.

Additional studies are needed to further clarify the mechanism of this activity and its potential in modulating the formation of LTs in atopic and asthmatic individuals both *in vitro* and *in vivo*. The results of these studies may be useful in developing new therapeutic approaches in asthmatic/allergic patients.

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