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Effect of alpha lipoic acid on leukotriene A_4 hydrolase

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A B S T R A C T

Leukotriene A_4 hydrolase is a soluble enzyme with epoxide hydrolase and aminopeptidase activities catalysing the conversion of leukotriene A_4 to leukotriene B_4 and the hydrolysis of the peptide proline-glycine-proline.

Imbalances in leukotriene B_4 synthesis are related to several pathologic conditions. Currently there are no available drugs capable to modulate the synthesis of leukotriene B_4 or to block its receptors. Here we show the inhibitory profile of alpha lipoic acid on the activity of leukotriene A_4 Hydrolase. Alpha lipoic acid inhibited both activities of the enzyme at concentrations lower than 10 µM. The 5-lipoxygenase inhibitor zileuton, or the 5-lipoxygenase activating protein inhibitor MK-886, were unable to inhibit the activity of the enzyme.

Acute promyelocytic leukaemia HL-60 cells were differentiated to leukotriene A_4 hydrolase expressing neutrophil-like cells. Alpha lipoic acid inhibited the aminopeptidase activity of the cytosolic fraction from neutrophil-like cells but had no effect on the cytosolic fraction from undifferentiated cells.

Docking and molecular dynamic approximations revealed that alpha lipoic acid participates in electrostatic interactions with K-565 and R-563, which are key residues for the carboxylate group recognition of endogenous substrates by the enzyme.

Alpha lipoic acid is a compound widely used in clinical practice, most of its therapeutic effects are associated with its antioxidants properties, however, antioxidant effect alone is unable to explain all clinical effects observed with alpha lipoic acid. Our results invite to evaluate the significance of the inhibitory effect of alpha lipoic acid on the catalytic activity of leukotriene A_4 hydrolase using *in vivo* models.

1. Introduction

Leukotriene A_4 hydrolase (LTA_4H, E.C: 3.3.2.6) is a zinc metalloenzyme that participates in the metabolism of lipid mediators and peptides (Haeggström et al., 2007; Appiah-Kubi and Soliman, 2015). This cytoplasmic enzyme is expressed mainly in myeloid cells specifically neutrophils, but is also expressed in other cell types including cancer cells (Chen et al., 2004). LTA_4H is a bifunctional enzyme showing two catalytic activities; aminopeptidase activity hydrolysing the tripeptide proline-glycine-proline (PGP, Fig. 1), and epoxide hydrolase activity on leukotriene A_4 (LTA_4, Fig. 1) generating leukotriene B_4 (LTB_4), a potent inducer of macrophage, T lymphocyte and neutrophil chemotaxis (Snelgrove et al., 2010). Therefore, this enzyme has two catalytic pockets, a hydrophobic domain that recognizes the lipophilic hydrocarbon chain of LTA_4 and a hydrophilic domain that recognizes the N-terminal region of PGP. Both domains converge in a common catalytic zone where the carboxylate group of each substrate interacts with alkaline amino acids in the protein. Thus, different catalytic pockets for separate activities share a carboxylate group recognition zone. (Haeggström et al., 1990; Medina et al., 1991). Recently, (Stsiapanava et al., 2014) provided structural support to selectively inhibit LTA_4 hydrolysis.

LTB_4 is a powerful chemoattractant agent related to inflammation, cardiovascular disease, rheumatoid arthritis and cancer (Bäck et al., 2014). On the other hand, PGP is an inflammatory peptide released from collagen by metalloproteases 8 and 9 both secreted by neutrophils, and its hydrolysis is mediated by LTA_4H. Therefore, this enzyme shows both pro and anti-inflammatory effects (Snelgrove, 2011; Wetterholm et al., 1991).

Pharmacological efforts have been made to inhibit leukotriene-

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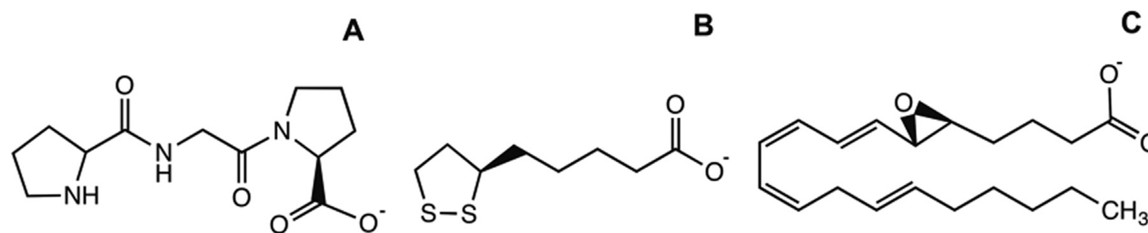

Fig. 1. Structures of A. Proline-glycine-proline, B. Alpha lipoic acid and C. Leukotriene A_4.

associated response. The drug zileuton, a 5-lipoxygenase inhibitor, showed satisfactory effects in asthmatic patients, however hepatotoxic effects have been reported (Watkins et al., 2007). At present the only clinically available agents are type 1 cysteinyl-leukotriene receptor blockers; montelukast, pranlukast and zafirlukast (Peters-Golden and Henderson, 2007). There are currently no drugs able to inhibit LTA_4H or to block LTB_4 receptors.

On the other hand, alpha lipoic acid ((R)-5-(1,2-dithiolan-3-yl) pentanoic acid; Fig. 1), an endogenous naturally synthetized compound, has shown several pharmacological effects related to anti-inflammation, cardiovascular disease, Alzheimer, bone loss, diabetic neuropathy and others. (Abdali et al., 2015; Huerta et al., 2016; Jiang et al., 2016; Roberts and Moreau, 2015; Serhiyenko and Serhiyenko, 2015; Skibska and Goraca, 2015). However its pharmacology is still not fully understood. Physiological functions of alpha lipoic acid are related to its role as a co-factor of pyruvate dehydrogenase complex, alpha ketoglutarate dehydrogenase, 2-oxoadipate dehydrogenase, branched-chain ketoacid dehydrogenase and the glycine cleavage system (Mayr et al., 2014). In addition, powerful antioxidant properties have been related with alpha lipoic acid acting as a metal scavenger (Zn^{2+}, Cu^{2+} and Hg^{+} mainly), through direct interaction with reactive oxygen species (ROS), and modulation of the redox homeostasis in the cell, etc. (Maczurek et al.; Moini et al., 2002; Rochette et al., 2013). However, the antioxidant effect of this compound by itself is not enough to explain all pharmacological properties associated to alpha lipoic acid.

Structural resemblance between LTA_4H substrates and alpha lipoic acid (Fig. 1), suggest that this compound could act as an inhibitor of the catalytic activity of LTA_4H. In this work, we show the inhibitory profile of alpha lipoic acid on the catalytic activity of LTA_4H.

2. Materials and methods

2.1. Materials

Alpha lipoic acid, L-alanine-p-nitroanilide, ninhydrin, thermolysin, casein, dimethylsulfoxide and others were purchased from Sigma-Aldrich Co. Inorganic acids and salts were purchased from Merck Co. Leukotriene A_4, recombinant human leukotriene A_4 hydrolase, proline-glycine-proline peptide, leukotriene B_4 EIA kit, zileuton, MK-886 and ultra-pure water were purchased from Cayman Chemical Co. RPMI 1640 culture media, fetal bovine serum, penicillin and streptomycin were purchased from Biological Industries.

2.2. Methods

2.2.1. Synthesis and characterization of methyl ester derivative of alpha lipoic acid (LAME)

To a solution of alpha lipoic acid (1.0 g. 2.2 mmol) in methanol under anaerobic conditions and provided with a Dean Stark system, three drops of concentrated sulfuric acid were added and the system was stirred at 78.3 °C for 1 h. The reaction was poured into a saturated aqueous solution of $NaHCO_3$ and transferred to a 250 ml separatory funnel. Subsequently the mixture was extracted with ethyl acetate (3×100 ml) and the combined organic phases were dried over anhydrous Na_2SO_4 and filtered. Solvent was removed by rotary evaporation under reduce pressure and the residue was purified by column chromatography on silica gel with CH_2Cl_2 as eluent to yield the target compound.

2.2.1.1. Methyl 5-(1,2-dithiolan-3-yl) pentanoate (LAME). Colorless, oil. Yield=95%. m.p oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.66 (s, 1 H), 3.56 (quintuplet, 1 H, J=6.4 Hz), 3.21-3.07 (m, 2 H), 2.45 (dtd, 1 H, J=12.0, 6.6, 5.4 Hz), 2.32 (t, 2 H, J=7.4 Hz), 1.90 (sextuplet, 1 H, J= 7.0 Hz), 1.73-1.61 (m, 1 H), 1.54–1.36 (m, 1 H). ^{13}C-NMR (400 MHz, $CDCl_3$) δ: 174.04, 56.45, 51.64, 40.34, 38.61, 34.72, 33.96, 28.88, 24.79. IR (KBr) cm^{-1}: 1736.51.

2.2.2. Epoxide hydrolase assay

Epoxide hydrolase activity was measured according to (Rao et al., 2007). Recombinant human LTA_4H (50 ng) was incubated with several concentrations of alpha lipoic acid, zileuton, MK-886 and LAME for 15 min at 37 °C in assay buffer (0,1 M potassium phosphate, pH 7.4, 5 mg/ml bovine serum albumin) in a volume of 50 µl. The solution was then adjusted to 200 µl with assay buffer and 10 µl of LTA_4 (final concentration 40 ng/ml) was added. After 10 min at 37 °C, the reaction was stopped by 20-fold dilution in assay buffer. LTB_4 was measured by enzyme immunoassay (EIA) according to manufacturer instructions (Cayman chem. N° 520111). The concentration of compounds necessary for half-maximal inhibition of enzyme activity (IC_{50}) was determined using non-linear regression in GraphPad Prism 7.0 (GrapPad).

2.2.3. Aminopeptidase assay using L-alanine p-nitroanilide

Aminopeptidase activity was measured using L-alanine p-nitroanilide as surrogate substrate as previously described (Rao et al., 2007). Recombinant human LTA_4H (500 ng) was incubated with several concentrations of alpha lipoic acid, zileuton, MK-886 and LAME for 15 min at 37 °C in assay buffer (50 mM Tris-HCl, pH 8.0, 100 mM KCl). An equal volume of 2-fold concentrated L-alanine p-nitroanilide was added to a final concentration of 1 mM in a volume of 110 µl. Aminopeptidase activity was determined by following the absorbance at 405 nm in a microplate reader Statfax 4200.

2.2.4. Measurement of PGP degradation: free proline determination

Recombinant human LTA_4H (500 ng) was incubated with several concentrations of alpha lipoic acid, zileuton, MK-886 and LAME for 15 min at 37 °C in assay buffer (50 mM Tris-HCl, pH 8.0, 100 mM KCl) in presence of 4 mM PGP. The reaction was stopped by 20-fold dilution in assay buffer. To 250 µl of this solution, glacial acetic acid (250 µl) was added, followed by 250 of ninhydrin solution (25 mg/ml in acetic acid/6 M phosphoric acid; heated at 70 °C to dissolve). The reaction mixture was heated at 100 °C during 60 min, allowed to cool to room temperature and the proline containing fraction extracted with 500 µl of toluene. Proline-ninhydrin conjugate was measured at optical density at 520 nm (Snelgrove et al., 2010).

2.2.5. Cell culture

Human promyelocytic leukaemia cells (HL-60) were cultured in RPMI medium supplemented with 10% fetal bovine serum, 100 UI/ml

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