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Effect of alpha lipoic acid on leukotriene A_4 hydrolase

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ABSTRACT

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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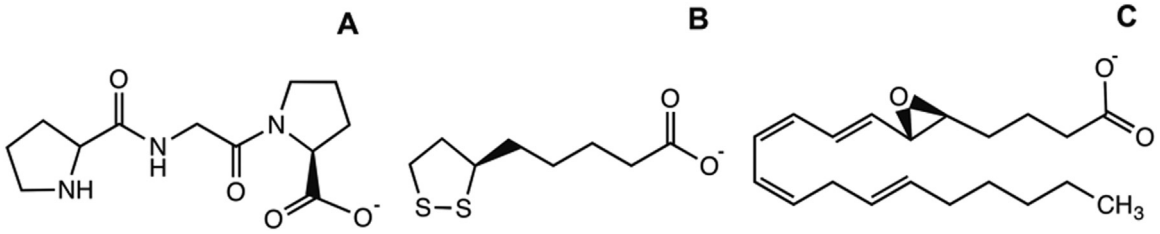

Fig. 1. Structures of A. Proline-glycine-proline, B. Alpha lipoic acid and C. Leukotriene A_4.

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

penicillin and 10 mg/ml streptomycin (complete media) in an incubator at 37 °C and 5% CO_2 (Scoggan et al., 1996).

2.2.6. Cell differentiation and cytosolic fraction

Neutrophil-like cells were obtained through HL-60 cells differentiation in complete media supplemented with 1.5% Dimethylsulfoxide (DMSO) for 5 days (Scoggan et al., 1996). Cytosolic faction was obtained by ultracentrifugation according (Letelier et al., 2006).

2.2.7. Measurement of theoretical data: docking and molecular dynamics

Alpha lipoic acid, proline-glycine-proline and leukotriene A_4 were constructed and the partial charges corrected using Spartan 14. Topology and parameters for the compounds were obtained using ParamChem server (Zoete et al., 2011). The molecular docking of the ligands with leukotriene A_4 hydrolase (PDB_{ID}: 3FTS) were done using AutoDock Vina. The volumes for the grid maps were 60×60×60 points (a grid-point spacing of 0.375 Å). The docked compound complexes were built using the lowest docked-energy binding positions. Once the complexes were obtained each pair protein-ligand was solvated with water model TIP3 and submitted to molecular dynamics (MD) simulations for 20 ns using a NPT ensemble. NAMD 2.10 was used to perform MD calculations. Periodic boundary conditions were applied to the system in the three coordinate directions. A pressure of 1 atm and a temperature of 310 K were maintained.

3. Results

3.1. Effect of alpha lipoic acid on epoxide hydrolase activity of LTA_4H

The epoxide hydrolase activity of recombinant human LTA_4H was potently and dose-dependently inhibited by alpha lipoic acid, with an IC_{50} value of 0.23 ± 0.1 μM (Fig. 2A and B). The inhibitory effect of alpha lipoic acid on the epoxide hydrolase activity of LTA_4H was compared with the 5-lipoxigenase inhibitor zileuton which has shown inhibitory effect on this enzyme (Landa et al., 2013; Rao et al., 2007), and the 5-lipoxygenase activating protein inhibitor MK-886. These compounds (10 μM) had no effect on epoxide hydrolase activity (Fig. 2C). In order to evaluate the role of the carboxylate group of alpha lipoic acid, we assessed the effect of alpha lipoic acid methyl ester (LAME) on LTB_4 production catalysed by human LTA_4H. LAME had no effect on the epoxide hydrolase activity of the enzyme at assayed concentrations (to 10 μM), Fig. 2C.

3.2. Effect of alpha lipoic acid on amino peptidase activity of human LTA_4H

The aminopeptidase activity of LTA_4H using L-alanine-*p*-nitroanilide as substrate was dose-dependently inhibited by alpha lipoic acid, with an IC_{50} value of 1.9 ± 0.2 μM (Fig. 3A and B). The general aminopeptidase inhibitor bestatine was used as positive control.

Recently, (Snelgrove et al., 2010) showed that the physiological substrate of the aminopeptidase activity in LTA_4H is the tripeptide proline-glycine-proline, a biomarker in chronic obstructive pulmonary disease (COPD). Fig. 3D shows that alpha lipoic acid inhibited proline release in a dose-dependent manner, with an IC_{50} value of 2.0 ± 0.2 μM (Fig. 2E). zileuton, MK-886 and LAME at 10 μM had no effect on the aminopeptidase activity of LTA_4H (Fig. 3C and F).

In addition, we evaluated the effect of alpha lipoic acid on thermolysin, a prototypic enzyme of the M family Zn^{2+}-dependent aminopeptidases to which LTA_4H belongs (Tholander et al., 2010). Alpha lipoic acid had no effect on the catalytic activity of thermolysin (not shown).

3.3. Effect of alpha lipoic acid on aminopeptidase activity of cytosolic fraction from human neutrophils

Human acute promyelocytic leukaemia cells (HL-60) were differentiated to neutrophils by addition of 1,5% dimethyl-sulfoxide (DMSO) to culture medium for 5 days (Scoggan et al., 1996). Neutrophil-like cells were lysed according to 2.2.6 and cytosolic aminopeptidase activity using L-alanine-*p*-nitroanilide as substrate was measured. Undifferentiated HL-60 cells were used as control. Alpha lipoic acid had no effect on cytosolic aminopeptidase activity of HL-60 cells (Fig. 4A), but dose-dependently inhibited the cytosolic aminopeptidase activity of differentiated HL-60 cells (Fig. 4B), indicating that alpha lipoic acid is only able to inhibit aminopeptidases expressed after a differentiating process induced by DMSO. Alpha lipoic acid concentrations higher than 100 μM (up to 1 mM, not shown) did not increase the percentage of inhibition any further (Fig. 4B). IC_{50} value was calculated considering the maximum effect observed at 100 μM and 2.3 ± 0.3 μM was obtained.

3.4. Study of interactions generated between alpha lipoic acid and LTA_4H

In order to obtain structural insights about the mode in which alpha lipoic acid and LTA_4H are interacting and the main interactions in the complex, docking and molecular dynamics simulations were performed.

The binding cavity of LTA_4H is formed by H-299, H-295, E-318 and Zn^{2+}. Thus, a positive charge region (R-563 and K-565) and a metal was observed. The molecular interactions study of the complex LTA_4H/alpha lipoic acid by docking showed a preference of the ligand to interact with the positive-charged amino acids R-563 and K-565 which is reasonable considering that the carboxylate group generate strong

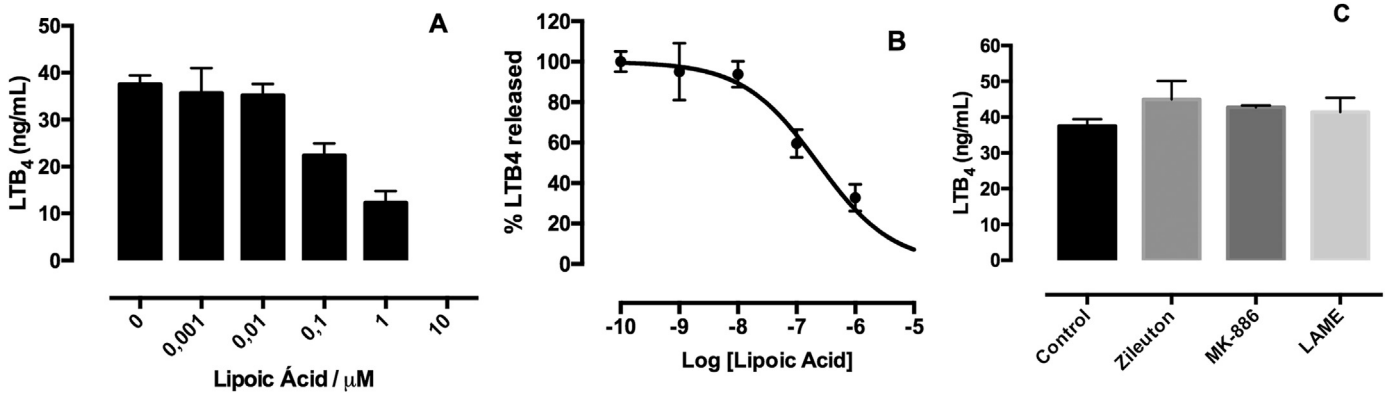

Fig. 2. Effect of alpha lipoic acid on epoxide hydrolase activity of LTA_4H. LTB_4 production was determined using enzyme immune assay (EIA). A. Effect of alpha lipoic acid on LTB_4 production. B. IC_{50} determination by non-linear regression using GraphPad Prism 7.0. C. Effect of zileuton, MK-886, and LAME on LTB_4 production.

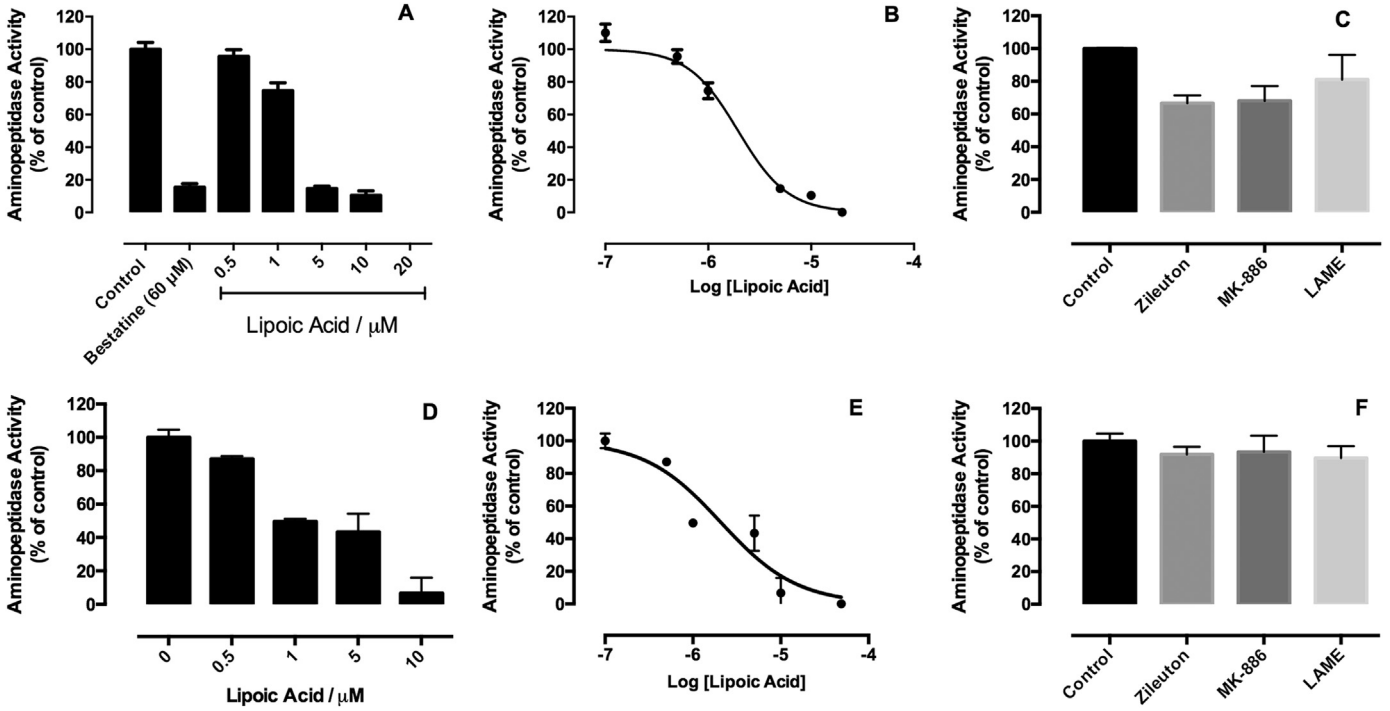

Fig. 3. Effect of alpha lipoic acid on aminopeptidase activity of LTA_4H. A-C; Activity was determined using L-alanine-p-Nitroanilide as substrate. C-F; Activity was determined using PGP as substrate. A and D; Effect of alpha lipoic acid on aminopeptidase activity of LTA_4H. B and D; IC_{50} determination by non-linear regression using GraphPad Prism 7.0. C and F; Effect of zileuton, MK-886, and LAME on LTB_4 production.

charge-charge interactions. Our results are supported on previous reports which inform that R-563 and K-565 in the C-terminal domain of LTA_4H are key residues involved in carboxylate recognition from endogenous substrates of the enzyme (Haeggström et al., 2007; Stsiapanava et al., 2014).

Fig. 5A shows that the carboxylate group of alpha lipoic acid situates at 3 Å from R-563 and 3 Å from K-565 in the catalytic pocket of the protein, suggesting that alpha lipoic acid participates in electrostatic interactions with these residues. Similar study and result were obtained with LTA_4 and PGP (not shown).

Once the complex protein-ligand was obtained the system was solvated and molecular dynamic simulations were carried out. This study allows us to understand the main interaction from both, a dynamic and an energetic point of view. The simulation results show that the system remains stable during the simulation (Fig. 5B: RMSD 2.0 Å). The analysis shows that electrostatic interactions between alpha lipoic acid and R-563 and K-565 were conserved at the studied time (20 ns) (Fig. 5C, D, E and F). However, at 7.5 ns carboxylate group of alpha lipoic acid rotate increasing its distance from K-565 and R-563, this facilitate the entry of water molecules. The solvation effect could be associated at a reversibility effect however in order to probe that experimental data is required (Fig. 5 C, D and G).

4. Discussion

It is known that leukotriene imbalances are related to several physiopathological conditions; in particular LTB_4 has been associated with asthma, cardiovascular disease, rheumatoid arthritis and cancer (Paige et al., 2014; Peters-Golden and Henderson, 2007; Yousefi et al., 2014). Here, we show that alpha lipoic acid inhibited both activities of LTA_4H (Figs. 2 and 3). The inhibition on LTB_4 synthesis was dose-dependent (IC_{50}=0.23 ± 0.1 μM), showing a full inhibition at concentration above 20 μM. Moreover, alpha lipoic acid inhibited the aminopeptidase activity of LTA_4H at the same range of concentrations (Fig. 3) suggesting that this compound can interact with the enzyme in a recognition common zone for both endogenous substrates. It is important to note that alpha lipoic acid inhibited the aminopeptidase activity of the enzyme regardless of the substrate used. Therefore,

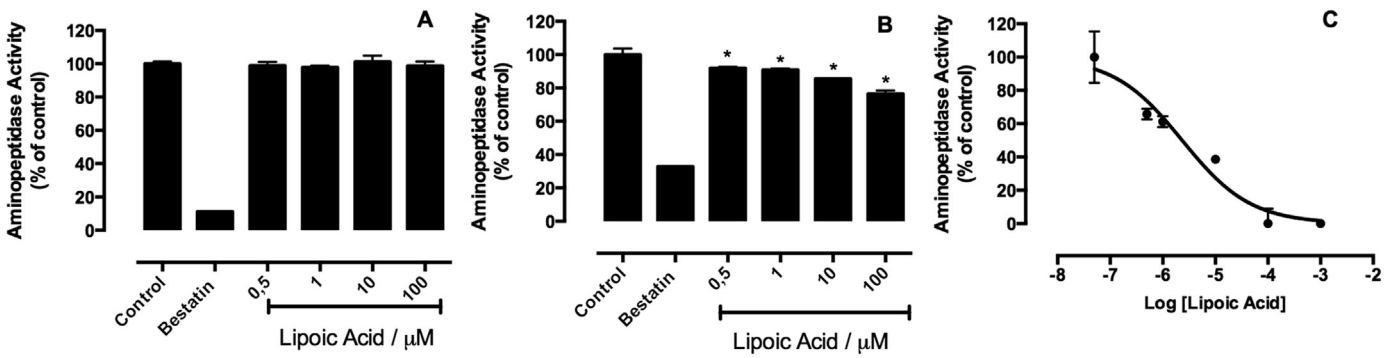

Fig. 4. Effect of alpha lipoic acid on aminopeptidase activity of cytosolic fraction from neutrophil-like cells. Aminopeptidase activity was determined using alanine-p-nitroanilide as substrate. A. Effect of alpha lipoic acid on cytosolic fraction from undifferentiated HL-60 cells, B. Effect of alpha lipoic acid on cytosolic fraction from neutrophil-like cells, C. IC_{50} determination by non-linear regression using GraphPad Prism 7.0.

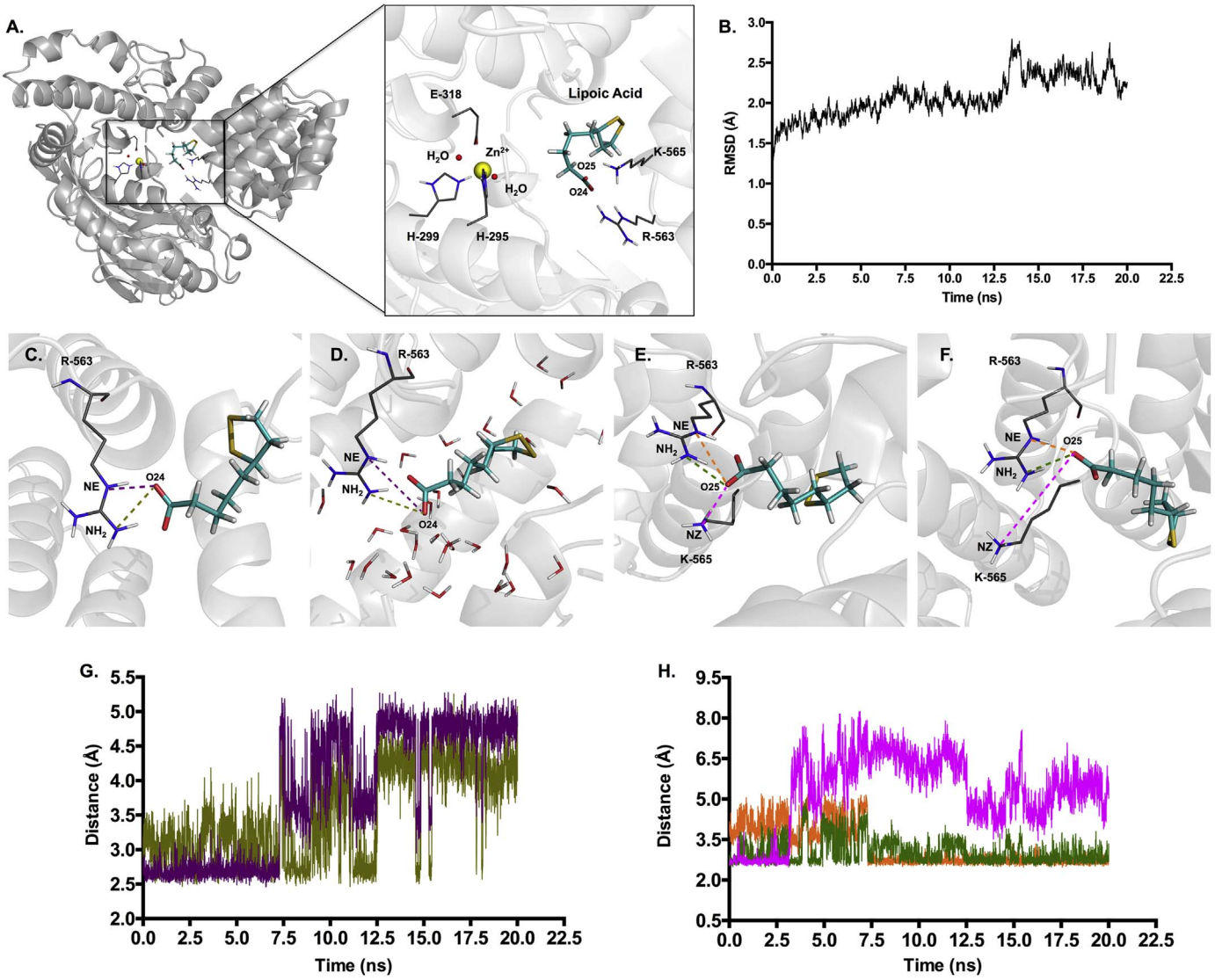

Fig. 5. Interactions generated between alpha lipoic acid and LTA_4H. A. Molecular coupling between 3D structure of human LTA_4H and alpha lipoic acid. Enzyme is represented in grey. The zoomed view shows the position of alpha lipoic acid in the catalytic pocket of the enzyme, represented by Zn^{2+} in interaction with amino acids residues. B. Time-depended RMSD, C-F. Alpha lipoic acid selected from the molecular docking studies to be subjected to molecular dynamic simulations. C and E at 2.5 ns and D and F at 10 ns. G. Distances between nitrogen NE and NH2 from R-563 and Oxygen (O24) from alpha lipoic acid. H. Distances between nitrogen NE and NH2 from R-563, nitrogen (NZ) from K-565 and Oxygen (O25) from alpha lipoic acid.

future experiments aimed to evaluate the effect of alpha lipoic acid on the aminopeptidase activity of the enzyme could be performed using either endogenous or surrogate substrates (Fig. 3).

Rao et al., 2007 has shown that the 5-lipoxygenase inhibitor zileuton is able to inhibit other enzymes related to leukotriene synthesis including LTA_4H (Rao et al., 2007). In our assays neither 10 μM zileuton or 10 μM MK-886 were able to inhibit LTA_4H, although inhibition may be observed at concentrations higher than 10 μM.

Human leukotriene A_4 Hydrolase is overexpressed in acute promyelocytic leukaemia cells exposed to DMSO for 5 days to induce cellular differentiation into neutrophil-like cell (Scoggan et al., 1996). Alpha lipoic acid showed inhibitory effect on the total aminopeptidase activity of cytoplasmic fraction from neutrophil-like cell and had no effect on aminopeptidase activity from undifferentiated cells (Fig. 4). Hence, alpha lipoic acid is able to inhibit the native LTA_4H and other aminopeptidases expressed after a differentiation process. The IC_{50} obtained on the cytosolic fraction from neutrophil-like cell was the same value obtained when a recombinant enzyme was assayed (~ 2 μM, Figs. 3B, E and 4E), and the inhibitory effect did not increase at concentrations greater than 100 μM (Fig. 4F). Additionally, we evaluated the effect of alpha lipoic acid on thermolysin, the prototype of the Zn^{2+}-aminopeptidases (Adekoya and Sylte, 2009) used in inhibitor effect evaluation. Recently, (Tholander et al., 2010) showed that the catalytic domain between LTA_4H and thermolysin are conserved. Alpha lipoic acid did not affect the aminopeptidase activity in all the range of concentrations assayed (up to 1 mM, not shown), these data together suggest that the inhibitory effect of alpha lipoic acid is selective for LTA_4H, however assays on other aminopeptidases are necessary.

In order to evaluate the carboxylate group relevance to the inhibitory effect displayed by alpha lipoic acid, we synthetized the methyl ester derivative of alpha lipoic acid (2.2.1). This modification abolished the inhibitory effect on both activities of the enzyme (Figs. 2C, 3C and F), indicating that the integrity of the carboxylate group in alpha lipoic acid is a key for its inhibitory effect and suggests that this group may participate in interactions with the enzyme at regions where endogenous substrates are recognized, thus substituting the carboxylate group can block those interactions. However, addition of the ester methyl group may result in steric effects that hinder the access of LAME into the catalytic pocket of the enzyme.

Aiming to study the interactions established between LTA_4H and

alpha lipoic acid, we performed docking and molecular dynamic approximations. Several reports support that residues R-563 and K-565 are crucial for recognition of the carboxylate group of endogenous substrates by the enzyme; mutations at any of these residues decrease or remove the catalytic activity of the enzyme (Rudberg et al., 2004; Thangapandian et al., 2012). Docking studies (Fig. 5A) showed that alpha lipoic acid may locate in the catalytic pocket of the enzyme and that its carboxylate group is orientated near both R-563 and K-565 (3 Å) establishing electrostatic interactions with these residues. These results explain the non-selective inhibition displayed by alpha lipoic acid on the enzyme and the abolishment of its inhibitory effect by the methyl group addition. Moreover, given that LAME was unable to inhibit the enzyme activity, simultaneous interactions between carboxylate group from alpha lipoic acid and R-563 and K-565 for a whole inhibitory effect are suggested.

Molecular dynamic studies revealed that electrostatic interaction between the carboxylate group of alpha lipoic acid and R-563 and K-565 are conserved throughout the time (20 ns) analysed. At 7.5 ns conformational changes of alpha lipoic acid favour the entry of water molecules into the cavity, increasing the distance and thus weakening the electrostatic interactions (Fig. 5C-H). This suggests that alpha lipoic acid remains in the catalytic pocket of the enzyme for a limited amount of time and thus a reversible inhibition is proposed. We are currently performing experiments directed to elucidate the inhibitory mechanism of alpha lipoic acid on LTA_4H.

Our results invite a study of potential therapeutic uses of alpha lipoic acid on pathologies where LTB_4 synthesis is upregulated. PGP is an inflammatory mediator in COPD, however, it is important to note that in COPD patients PGP is been acetylated and acetyl-PGP is not substrate of LTA_4H (Snelgrove et al., 2010). Nevertheless, the extent of pro-inflammatory and anti-inflammatory effect mediated by LTA_4H inhibition should be evaluated using *in vivo* models.

Alpha lipoic acid after an oral dose of 500 mg induces plasmatic concentration of 50 μM (Bilska and Włodek, 2005; Shay et al., 2009). In our experiments alpha lipoic acid inhibited the catalytic activity of LTA_4H at concentrations lower than 10 μM, making it possible to think that this effect could be observed with *in vivo* models.

The non-selective inhibition of LTA_4H by alpha lipoic acid can help to explain some of its pharmacological effects non-related to its antioxidant properties but to an anti-inflammatory profile, as diabetes, multiple sclerosis and anti-inflammatory agent (Shay et al., 2009), increasing the pharmacological knowledge of this molecule. On the other hand, several diseases have been associated with increments in LTB_4 concentrations including cardiovascular diseases and cancer (Chen et al., 2004; Mathis et al., 2007; Subbarao et al., 2004). LTB_4 participates in atherosclerotic lesions, contributing to monocyte recruitment and foam-cell differentiation (Aiello et al., 2002; Peters-Golden and Henderson, 2007). Likewise, incidence of stroke and myocardial infarction has been linked to overproduction of LTB_4 and LTA_4H has shown a positive role in angiotensin-dependent cardiac inflammation in hypertension (Letts, 1987; Peters-Golden and Henderson, 2007). In cancer, LTB_4 signalling is associated to malignant transformation and neoplastic cell proliferation (Çalışkan and Banoglu, 2012; Chen et al., 2004). Alpha lipoic acid supplementation has shown a beneficial effect reducing the incidence of hypertension, atherosclerosis and heart failure (Abdali et al., 2015; Bilska and Włodek, 2005; Huerta et al., 2016). In addition, several reports have shown antitumor effects of alpha lipoic acid (Dörsam and Fahrer, 2016; Schwartz et al., 2010), and according to our results these effects might be mediated by its inhibitory effect on LTA_4H.

Given the structural resemblance between alpha lipoic acid and leukotriene A_4 is highly likely that alpha lipoic acid inhibits other proteins whose endogenous substrate is leukotriene A_4 such as Leukotriene C_4 synthase and microsomal glutathione S-transferase-2. Currently our laboratory is assaying the effect of alpha lipoic acid on these enzymes.

Up to date, there are no drugs able to inhibit LTA_4H and given that alpha lipoic acid is an approved compound for human use, weakly toxic and with more than 60 years of clinical use, clinical assays to evaluate the clinical significance of *in vitro* inhibition of LTA_4H by alpha lipoic acid could be suggested.

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