Research Article

Antiallergic activity of alpha-lipoic acid

Vaishnavi Sivakali Subramanian, V. Vishnu Priya*, R. Gayathri

ABSTRACT

Aim: The goal of the present study is to determine the alpha-lipoic acid (ALA) prevents the release of beta-hexosaminidase enzyme and proves its antiallergic activity. **Materials and Methods:** RBL-2H3 cells were cultured in minimum essential medium supplemented. The cells were seeded in 24-wells plate (5 × 105 cells/mL) and incubated to adhere at 37°C in 5% CO_2 for 1.5 h. RBL-2H3 cells were sensitized with anti-DNP immunoglobulin E and incubated at 37°C in 5% CO_2 for 24 h. The ALA with different concentrations (20, 40, 80m and 100 µg/ml) was dissolved in media and used for the further test. The cells were washed with 400 µL of Siraganian buffer (buffer A). The test sample (20 µL) solution was added to each well and incubated for 10 min, followed by addition of 20 µL of antigen at 37°C for 20 min. The supernatants were transferred into 96-well plate in 50 µL/wells and incubated with 50 µL of substrate p-nitrophenyl- N-acetyl-b-D-glucosaminide (PNAG) at 37°C for 3 h. The reaction was stopped by adding 200 µL of stop solution (0.1 M Na_2CO_3/$NaHCO_3$, pH 10.0). The absorbance was measured with a microplate reader at 405 nm. **Results:** Zone of inhibition for different concentrations of ALA increases with concentration with the highest zone of inhibition was seen with the positive control (Chlorpheniramine). Therefore, inhibition of beta-hexosaminidase enzyme is seen with ALA. **Conclusion:** Hence, ALA inhibits beta-hexosaminidase enzyme which is responsible for allergic and anaphylactic reactions. Although the inhibition is not up to the mark as in chlorpheniramine, further researches with higher concentrations of ALA can be done to evaluate the efficiency of antiallergic activity as equal as chlorpheniramine.

KEY WORDS: Alpha-lipoic acid, Antiallergic activity, Beta-hexosaminidase

INTRODUCTION

Alpha-lipoic acid (ALA), or 1,2-dithiolane-3-pentanoic acid, is a naturally occurring dithiol compound synthesized enzymatically in the mitochondrion from octanoic acid. Lipoic acid (LA) is a necessary cofactor for mitochondrial α-ketoacid dehydrogenases, and thus, serves a critical role in mitochondrial energy metabolism. LA has been described as a potent biological antioxidant, a detoxification agent, and a diabetes medicine; it has been used to improve age-associated cardiovascular, cognitive, and neuromuscular deficits and has been implicated as a modulator of various inflammatory signaling pathways.[1-8] Mast cells are bone marrow-derived effector cells of the immune system found abundantly in connective tissue, skin, mucosal membranes, and tissues which interface with the external environment. It has been thought that mast cells play a major role in the development of many physiological changes during allergic responses. Mast cell activation by both immunoglobulin E (IgE)-dependent and IgE-independent stimuli initiate degranulation which results in the fusion of cytoplasmic granule membranes with plasma membranes. This process is accompanied by the fast external release of granule-associated stored mediators (histamine, neutral proteases, acid hydrolyases, proteoglycans, chemotactic factors, and cytokines such as tumor necrosis factor-α and interleukins) as well as the generation and release of newly generated mediators, including products of arachidonic acid metabolism and an array of cytokines.[9] Histamine, a major component of mast cell granules, exerts many effects related to the immediate-phase of allergic inflammation including vasodilation, increased vascular permeability, and tissue edema.[10] Histamine is the main cause of many of the symptoms of allergies, such as the runny nose, sneezing, and itching. Histamine also contributes to the progression of allergic-inflammatory responses by enhancement of the secretion of proinflammatory cytokines.[11-13]

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Received on: 08-12-2018; Revised on: 12-01-2019; Accepted on: 04-02-2019

MATERIALS AND METHODS

Source of Chemicals

The ALA, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich company.

Cell Culture

RBL-2H3 cells were cultured in minimum essential medium supplemented with 15% fetal bovine serum, penicillin (100 units/mL), and streptomycin (100 µg/mL). The cells were seeded in 24-wells plate (5 × 105 cells/mL) and incubated to adhere at 37°C in 5% CO_2 for 1.5 h. RBL-2H3 cells were sensitized with anti-DNP IgE (anti-dinitrophenyl-IgE) (0.45 µg/mL), and incubated at 37°C in 5% CO_2 for 24 h.

Test Drugs

The ALA with different concentrations (20, 40, 80, and 100 µg/ml) was dissolved in media and used for the further test.

Determination of Antiallergic Activity

The cells were washed with 400 µL of Siraganian buffer (buffer A) (119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM $MgCl_2$, 1 mM $CaCl_2$, 25 mM piperazine-N, N′-bis(2-ethanesulfonic acid), 0.1% bovine serum albumin (BSA), and 40 mM NaOH, pH 7.2). An aliquot (160 µL) of buffer A was added and incubation was continued for an additional 10 min at 37°C. The test sample (20 µL) solution was added to each well and incubated for 10 min followed by addition of 20 µL of antigen (DNP-BSA, final concentration 10 µg/mL) at 37°C for 20 min to stimulate cell degranulation. The supernatants were transferred into 96-well plate in 50 µL/wells and incubated with 50 µL of substrate PNAG (1 mM p-nitrophenyl-N-acetyl-b-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37°C for 3 h. The reaction was stopped by adding 200 µL of stop solution (0.1 M Na_2CO_3/$NaHCO_3$, pH 10.0). The absorbance was measured with a microplate reader at 405 nm. The test samples were dissolved in DMSO and Siraganian buffer was added for dilution (final DMSO concentration was 0.1%). The positive controls showed a clear yellow color, whereas the negative control was colorless. The samples were pale yellow to colorless, representing the percentage of inhibition antiallergic activity. Chlorpheniramine was used by positive controls.

Statistical Analysis

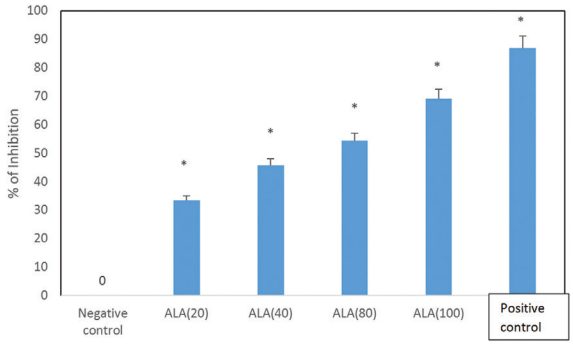
Results were expressed as mean ± SD. Statistical significance was determined by one-way analysis of variance and *post hoc* least-significant difference test. $P < 0.05$ was considered a statistically significant.

RESULTS AND DISCUSSION

Antiallaergic activity of ZAE

Treatment	Conc. (µg/ml)	Abs 405 nm Mean±SD
Negative control	-	0.428±0.28
ALA	20	0.285±0.15*
	40	0.232±0.11*
	80	0.195±0.14*
	100	0.132±0.11*
Positive control	100	0.056±0.04*

Values are expressed as Mean±SD (n=3); *P<0.05 statistically significant as compared with negative control. ALA: Alpha-lipoic acid, SD: Standard deviation



The graph depicts the beta-hexosaminidase release-inhibitory activity at various concentrations. Values are expressed as Mean ± SD (n = 3); *P < 0.05 statistically significant as compared with negative control. IC_{50} of antiallergic activity is 7 µM.

Zone of inhibition for different concentrations of ALA increases with concentration with the highest zone of inhibition was seen with the positive control (Chlorpheniramine). Therefore, inhibition of beta-hexosaminidase enzyme is seen with ALA.

Anti-allergic effects of ALA have not been reported despite the clinical usage as an anti-allergic agent. The effect of the extract on the immediate phase response will be attributed to the anti-degranulation from RBL-2H3 cells. The inhibitory effect on the release of β-hexosaminidase from RBL-2H3 cells will be evaluated according to the methods of Murata *et al*.

A study has demonstrated that LA inhibits compound 48/80-induced systemic anaphylaxis-like and passive cutaneous anaphylaxis-like reactions in mice. Pretreatment with the same concentration of LA before the systemic anaphylaxis-like reaction also reduces the plasma histamine content in a dose-dependent manner. Compound 48/80-induced histamine release from rat peritoneal mast cells (RPMCs) was blocked by treatment with LA. Compound 48/80 is well-known to induce mast cell dependent, non-specific anaphylactic reactions. It is widely accepted that the mechanism involved in the anaphylaxis-like response activated by compound 48/80 is due to the massive release of histamine from mast cells and basophils.

Therefore, these results suggest that LA could inhibit the mast cell-derived anaphylactoid reactions by preventing histamine release from RPMCs triggered by compound 48/80.[14,15]

CONCLUSION

Hence, ALA inhibits beta-hexosaminidase enzyme which is responsible for allergic and anaphylactic reactions. Although the inhibition is not up to the mark as in chlorpheniramine, further researches with higher concentrations of ALA can be done to evaluate the efficiency of antiallergic activity as equal as chlorpheniramine.

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Source of support: Nil; Conflict of interest: None Declared