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Dihydrolipoic but not alpha-lipoic acid affects susceptibility of eukaryotic cells to bacterial invasion

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ABSTRACT

Sensitivity of eukaryotic cells to facultative pathogenes can depend on physiological state of host cells. Previously we have shown that pretreatment of HeLa cells with N-acetylcysteine (NAC) makes the cells 2–3-fold more sensitive to invasion by the wild-type *Serratia grimesii* and recombinant *Escherichia coli* expressing gene of actin-specific metalloprotease grimelysin [1]. To evaluate the impact of chemically different antioxidants, in the present work we studied the effects of α-Lipoic acid (LA) and dihydrolipoic acid (DHLA) on efficiency of *S. grimesii* and recombinant *E. coli* expressing grimelysin gene to penetrate into HeLa and CaCo cells. Similarly to the effect of NAC, pretreatment of HeLa and CaCo cells with 0.6 or 1.25 mM DHLA increased the entry of grimelysin producing bacteria by a factor of 2.5 and 3 for the wild-type *S. grimesii* and recombinant *E. coli*, respectively. In contrast, pretreatment of the cells with 0.6 or 1.25 mM LA did not affect the bacteria uptake. The increased invasion of HeLa and CaCo cells correlated with the enhanced expression of E-cadherin and β-catenin genes, whereas expression of these genes in the LA-treated cells was not changed. Comparison of these results suggests that it is sulfhydryl group of DHLA that promotes efficient modification of cell properties assisting bacterial uptake. We assume that the NAC- and DHLA-induced stimulation of the E-cadherin-catenin pathway contributes to the increased internalization of the grimelysin producing bacteria within transformed cells.

1. Introduction

α-Lipoic acid (LA) known also as thioctic acid is a fatty acid widely distributed in cellular membranes, cytosol, and extracellular spaces. It is synthesized within the cells but mostly absorbed from food and transported to cytoplasm via fatty acid-, Na + -dependent vitamin- or H + -linked monocarboxylate-transporter systems [2–4]. Being a naturally occurring cofactor for the mitochondrial dehydrogenases LA is also involved in many other biological functions [5]. In cells LA reduces sulfhydryl groups with NADPH-dependent reductase producing the reduced form, dihydrolipoic acid (DHLA). Both the oxidized and reduced forms of LA show antioxidant properties. These compounds are able to chelate metals inhibiting hydroxyl radical production, act as a scavenger of reactive oxygen and nitrogen species. DHLA as a more powerful antioxidant is able to restore superoxide anion radical, regenerate endogenous antioxidants (vitamins C, E, glutathione) and restore oxidative injures [6,7], thus being deeply involved in the maintenance of the redox status of the cells.

Besides their direct antioxidant effects, the thiol-containing antioxidants interact with regulatory and signaling proteins modulating signal transduction and gene expression [8,9]. LA was shown to down-regulate expression of genes encoding redox-sensitive pro-inflammatory proteins and to induce expression of antioxidant genes [10]. It also inhibited expression of intercellular adhesion molecule-1 ICAM-1 [11] and alpha4beta1 integrin [12] and beta1-integrin [13]. The effects of other well-known thiol-containing antioxidant, N-acetylcysteine (NAC) on up- and down-regulation of genes responsible for cell proliferation and differentiation were studied in detail [14,15]. These data indicate that despite the difference of their chemical properties LA/DHLA and NAC can induce similar responses. To address this issue, LA-, DHLA- and NAC-induced effects should be compared in the same biological system.

We have previously shown that pre-treatment of HeLa cells with N-acetylcysteine increases their susceptibility to invasion by facultative pathogen *Serratia grimesii* producing actin-specific protease grimelysin and recombinant *Escherichia coli* expressing

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grimelysin gene [1]. This effect did not correlate with changes in glutathione level [16] or cytoskeleton rearrangements [1] but was accompanied by an increased expression of E-cadherin, cell surface receptor playing a role in cell adhesion and cell–cell junctions [1]. Taking these effects as a reference, in the present work we have tried to modify susceptibility of HeLa and CaCo cells to bacterial invasion by LA and DHLA in order to evaluate the impact of these chemically different compounds on the mechanisms of bacteria-host cell interaction.

Our results show that, similarly to NAC, DHLA increases susceptibility of HeLa cells to invasion by S. grimesii and up-regulates expression of E-cadherin gene. In contrast, no effect of LA on these properties was detected. These results show that the sulfhydryl activity of DHLA, like that of NAC, plays a role in the enhanced susceptibility of HeLa cells to bacteria. It is plausible that these reagents interact with cell surface receptors and either activate them directly or modulate signal transduction regulating the E-cadherin-β-catenin pathway.

2. Materials & methods

2.1. Reagents

Culture media αMEM and DMEM were obtained from Biolot LLC (Russia). Fetal bovine serum (FBS) was from Thermo Scientific (Thermo Fisher Scientific Inc.). Peptone and yeast extract were obtained from Difco (Franklin Lakes, NJ), nonessential amino acids (NEAA), NAC, PBS, sodium deoxycholate, Triton X-100, reagents for PCR, were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Cell cultures, bacterial strains, and growth conditions

Human cervical carcinoma HeLa M and colorectal adenocarcinoma CaCo-2 cells were obtained from the Russian Cell Culture Collection (Institute of Cytology, St. Petersburg, Russia). Cells were grown in the antibiotic-free αMEM supplemented with 10% FBS and 1% NEAA at 37 °C under 5% CO_2 atmosphere. *S. grimesii* strain 30063 was from the German Collection of Microorganisms and Cell Cultures (DSMZ). The recombinant *E. coli* SCS1, expressing grimelysin gene was obtained as described previously [30]. Bacteria were grown in Luria broth (LB medium) containing 1% peptone, 0.5% yeast extract, and 1% NaCl at pH 7.0 at 37 °C with aeration.

2.3. Incubation of cells with antioxidants

LA and DHLA were freshly dissolved in the culture medium. Cells were grown to get 60% confluents in 6-well plates for 24 h, and LA or DHLA was added to the medium to the final concentration of 0.6 or 1.25 mM for 22–24 h. After the medium was replaced by the fresh one without LA/DHLA the cells were used for quantitative invasion assay either immediately or after growing for 24 h more.

Cells were incubated with NAC as discribed previously [1].

2.4. Gentamicin invasion assay

Efficiency of invasion was evaluated by gentamycin invasion assay as discribed previously [17] with minor modifications [1].

2.5. RNA analysis

Isolation of RNA. For RNA isolation, the TRIZOL reagent (Invitrogen) was used following the manufacturer's instructions. Quality and quantity of isolated nucleic acids were estimated by spectrophotometry and gel electrophoresis.

cDNA preparation. For reverse transcription, 2 μg total RNA was reverse transcribed with oligo-dT (Invitrogen) and 200 U Moloney murine leukemia virus reverse transcription (Invitrogen) according to the manufacturer's instructions [32].

2.6. Real-time RT-PCR

Real-time RT-PCR was performed to examine the E-cadherin mRNA levels in Hela M, and CaCo-2 cells. Q-RT-PCR was performed using IQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) and an iCycler Thermal Cycler (Bio-Rad Laboratories). The quantity of mRNA was normalized to the housekeeping gene β-microglobulin.

2.7. Semi-quantitative RT-PCR

Expression analyses of E-cadherin gene was conducted by semiquantitative RT-PCR. Gene-specific primer pairs were designed using BLAST primer software and were as followed: β-Actin forward: 5′-CTG GGA CGA CAT GGA GAA AA-3′, β-Actin reverse: 5′-AAG GAA GGC TGG AAG AGT GC-3′, E-cadherin forward: 5′-CCC TGG CTT TGA CGC CGA GA-3′, E-cadherin reverse: 5′-CTC GGT CCA GCC CAG TGG TG-3′, β-microglobulin forward: 5′- GCT ATC CAG AAA ACC CCT CAA -3′, β-microglobulin reverse: 5′- CAT GTC TCG ATC CCA GTA GAC GGT -3′

PCR reactions were optimized to 94 °C for 3 min, 35 amplification cycles at 94 °C for 1 min, the appropriate annealing temperature (60 °C for β-actin and β-microglobulin and 64 °C for E-cadherin) for 1 min, 72 °C for 1 min, and a final extension of 10 min at 72 °C. Amplified products were resolved on 1.5% agarose gels and visualized by ethidium bromide staining.

2.8. Western blot

Cells were grown to confluence in plastic dishes, treated with antioxidants and then disrupted by a freezing–thawing procedure at −70 °C, further incubated for 10 min with buffer contained 5 mM Tris–HCl pH 7.5, 1 mM EDTA, 0.05% Nonidet P-40, 1 mM DTT, 1 mM PMSF, and protease inhibitor cocktail (Complete™, Roche Molecular Biochemicals).The proteins were separated by SDS-PAGE, and then transferred onto PVDF membranes. After been blocked with 1% BSA for 2 h at room temperature (RT), the membranes were then incubated with antibodies against E-cadherin (1:250, Abcam), and β-actin (1:1000, Abcam) at 4 °C overnight. After washing 3 times with TBST, the membranes were incubated with alkaline phosphatase-conjugated secondary antibody (1:10,000, Sigma–Aldrich) at RT for 2 h. Then washed the membranes 3 times with TBST and imaged with a gel imaging system (BIO-RAD).

2.9. Statistical analysis

Data were analyzed statistically using one-way analysis of variance (ANOVA) with Excel Data Analysis Pack. A difference was considered significant at the $p < 0.05$ level.

3. Results

3.1. α-Lipoic acid does not change susceptibility of HeLa and CaCo-2 cells to invasion by S. grimesii and recombinant E. coli expressing grimelysin gene

Pre-treatment of HeLa cells with 10 mM N-acetylcysteine increased the entry of grimelysin producing bacteria by a factor of 1.5–2 and 3-3.5 for wild-type *S. grimesii* and recombinant *E. coli* expressing grimelysin gene, respectively [1]. To evaluate

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