Original Article

Role of Specific Bioactive Fraction of Rhodiola Rosea in Combination with Lipoprotein Fraction from Trachurus Against Oxidative Stress in Caulobacter Cresenctus

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Abstract

Introduction: Aging process is associated with the accumulation of free radicals that tilts cellular homeostasis leading to cellular senescence. The Specific Bioactive Fraction (SBF) from Rhodiola plant in combination with a class of lipoproteins (LF) derived from the fish Trachurus sp contains bioactive compounds mainly rhodioloside or salidroside which has capability to quench oxidative stress and exhibits anti-inflammatory effects with immuno-enhancing and modulating properties. Hence, in this study we tested the bioactive compound (R-L) in Caulobacter cresentus against induced oxidative stress

Methods: The bacterial cells were treated with different concentration of R-L bioactive compound to obtain the minimum lethal dosage. The bacteria was administered with hydrogen peroxide to induce oxidative stress and thereby aging. Various anti-aging and biochemical assays were carried out like LPO, Catalase, SOD, GSH, Elastase and Beta galactosidase.

Results: In cells treated with hydrogen peroxide, more Beta Galactosidase activity was observed, which confirms they were undergoing senescence, which was reversed by treatment of R-L compound. Similarly other antioxidant assays like LPO, SOD exhibited significant downregulation after R-L compound treatment.

Conclusions: Our study displays that hydrogen peroxide treatment induced senescence in cultured Caulobacter cresentus, likely from oxidative stress. The bioactive compounds usually have anti-oxidant and anti-aging properties that can prevent the cells by the damages caused due to intrinsic and extrinsic factors was evident by the results of assays such as lipid peroxidase, catalase, superoxide dismutase, elastase. However, additional studies are yet to be done to confirm the anti-aging effects of the bioactive compound.

Keywords: Caulobacter cresentus, Rhodiola LF-T, Rhodiola SBF+LF-T(R-L compound), Reactive oxygen species, Cellular senescence.

Introduction

Lower levels of Reactive oxygen species (ROS) production are required to maintain normal physiological functions, including host defense, proliferation, and continuing homeostasis. However, inconsistent generation of ROS poses a severe problem to homeostasis and thereby leads to oxidative tissue damage. There are already existing theories of role of oxidative stress in aging as well as in age-related diseases. This theory specifies the aging as a series of process that leads to the accumulation of the free radicals which eventually leads to imbalanced homeostasis which ultimately induce cellular senescence. Aging is a complex biological and time-dependent phenomenon which is depicted by the deterioration of the structural and functional decline of the cells progressively.[1] Excessive (ROS) formation can induce oxidative stress leading to the cell damage that can result in cellular senescence. Therefore, the cells have antioxidant network to scavenge excessively produced ROS. The anti-oxidants can attenuate the damaging effect of ROS and delay many events that contribute to cellular aging. The aging process leads to the disorders including chronic, pro-inflammatory signals which cause risk towards developing aging-related disorders (ARDs), impairment in the regenerative ability of the stem cells.[2]

In recent years, antioxidants are commonly used as the provision to delay aging and are found in the dietary supplements.[3] The anti-oxidant defense system includes endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione (GSH), and coenzyme Q. Since, oxidative damage of cell increases with the age, the increased intake of exogenous antioxidants from fruits, vegetables and other plant extract may support the endogenous antioxidant defense system of the cell.

The recent technological advancement aims at explicit purpose of delaying the aging process which would prolong a healthy life. The current research in gerontology is to develop medications that are capable to modulate the aging process. In this regard, plant extracts and many novel phyto-marine compounds are being used now a days to reduce oxidative stress. The anti-oxidant potency of a plant extract depends upon the presence of phenolic compounds in the extract.[4] One such plant often administered for enhancing immune functions is Rhodiola rosea. Parts of Rhodiola belongs to plants revealing adaptogenic properties, which are attributed to the presence of specific phenolic compounds and are reflected mainly as antioxidant activity. A wide variety of preclinical in vivo and ex vivo studies have elucidated the presence of several biochemical and pharmacological stress reducing actions of Rhodiola.[5] In this investigation we sought to test the efficacy of a special bioactive fraction from Rhodiola (SBF) combined with lipoprotein from Trachurus sp fish (LF-T) which may actively related with its biological activity. Additionally, the lipoprotein fraction was isolated from Trachurus species (denoted as LF-T) which is reported to exhibit anti-inflammatory effects with immuno-enhancing and modulating properties.[6] During the experiment, we exposed the bacteria Caulobacter crescents with H_2O_2 to induce oxidative stress. The bacterial species was specifically selected since it is well known as a model to study oxidative stress and age related disorders.[7-9]

Materials and Methods

Bacterial Strain

The Caulobacter cresentus bacterial strain were procured from Microbial Type Culture Collection and Gene Bank, MTCC No. 7510T in the freeze dried form in a vial which was used for all the bacteriological experiments and the culture was stored in 4°C and preserved for further use.

Caulobacter Cresecentus as the model of studying oxidative stress and aging process

Caulobacter cresentus is a model for the aging and the first bacterium reported to exhibit replicative aging in which cytokinesis is intrinsically asymmetrical. The bacteria have ability to respond to oxidative stress by destabilizing and inactivating proteins invoking peroxide and superoxide molecules.[7-9]

Bacteria culture and growth condition

Bacterial cells were grown in PYE medium at 37°C for 18 hours. Those cells were grown on PYE Agar in petri plates at 37°C. After the formation of the visible colonies, plates were taken out of the incubator and kept at 4°C. All the experiments were performed using cultured bacterial cells.

Preparation of R-L (combination of Extract from Rhodiola and L-F of Trachurus sp) Compound

Rhodiola SBF and LF-T (R-L compound) was a kind gift from ReGenera R&D International for Aging Intervention, Milano, Italy. The combinational compound was obtained from the company and the specific formulation was not disclosed under the Copyright Act of the company. The R-L compound was used for further treatments in Caulobacter crescentus.

Preparation of Stock Solution

0.01g of R-L compound was dissolved in 9ml Dulbecco's Modified Eagle Medium (DMEM) and 1ml of fresh Dimethyl Sulfoxide (DMSO) was prepared and stored in room temperature

Preparation of Working Solution

0.1ml of R-L compound solution was prepared using 10ml Phosphate Buffer Saline (PBS) and mixed well and the solution was stored at room temperature. Various concentrations of R-L compound were made and used for further experiments.

Cell viability

Kirby Bauer Assay

The Kirby Bauer Assay perform to check antibiotic sensitivity of bioactive compound. The cells were grown up to log phase and spread by using cotton swab techniques. Different paper disks infused with different concentration of R-L compound and H_2O_2 after that placed over culture containing agar in plate incubated at 37°C for 18 hours.

Preparation of Bacterial Cell suspension

Specific dose of H_2O_2 and the treated bacterial cells in the concentration of 2ng/ml (R-L1) and 100ng/ml (R-L2) compound were collected and processed and the cell lysate was stored at 4°C for further assays listed below.

Protein estimation by Lowry's method

The Lowry's method performed for total protein estimation was followed and absorbance was meas-

ured at 660 nm wavelength with Folin-Ciocalteau reagent. After the assay as per standard protocol, the absorbance of the unknown sample was taken and the concentration was determined using the standard curve plotted.

Biochemical assays

Lipid peroxidase assay:

The degradation of lipids in cell membrane refers to the lipid peroxidation. The Malonydialdehyde (MDA), a product of Lipid peroxidation form pink color complex when heated with Thiobarbituric acid. 1ml of cell lysate was mixed with 2ml of TCA-TBA-HCL reagent in a centrifuge tube and heated for 15 minutes in a boiling water bath. After cooling, the mixture was centrifuge at 1000 rpm for 10 minute to remove the precipitate. Milli-Q water used as a blank. The absorbance of the sample was measured at 535nm. The activity was calculated and expressed as µM /min/mg protein.

Catalase assay

Catalase enzyme convert hydrogen peroxide into less reactive gaseous oxygen and water molecule in the cell. The 3 ml of reaction mixture was prepared which contains 50mM phosphate buffer 5mM H_2O_2 and 0.1ml cell lysate. The absorbance was measured at 240nm up to 3 minutes at 30 seconds interval.The activity was calculated and expressed as µM /mg protein/min.

Super oxide dismutase assay (SOD)

The Superoxide dismutase enzyme catalases the disputation of superoxide into oxygen and hydrogen peroxide in the cell. The SOD is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto oxidation. The optical density was measured at 420 nm against Tris-EDTA buffer per minute of the addition of pyrogallol and concentration of SOD was expressed as µM /mg protein.

Total thiol content assay

The cell lysate of 0.65ml was mixed with 0.1ml of 4% Sulfosalicylic acid, 0.5ml of 0.1M Sodium Phosphate Buffer and the mixture was made up to 2.5ml with 1.25 ml of Milli-Q water. To the 1 ml of the test solution were added 6.6 µl DTMB and the control sample without DTNB from the experiment. The tubes were allowed to stand for 1hour and 15 min and then vortexed for 15 seconds. The absorbance was taken at 412nm and the values were calculated and expressed as µM/ml of cell suspension.

Anti-aging assays

Elastase Assay

The elastase inhibitory activity was measured by the modified method of Sigma Aldrich and Thring et al., (2009). In 3.00ml reaction mix, the final concentrations is 96.7 mM Trizma, 0.29mM N-Succinyl-Ala-Ala-Ala-p-nitro anilide and 0.02-0.05 unit of elastase and mixed by inversion and equilibrate to 25°C and the absorbance was taken at 410 nm.

Beta-Galactosidase Assay

The activity of beta Galactosidase was tested using Beta Galactosidase Assay Kit (Medox-Bio, Cat No.MX1542-10) according to the kit instruction for the treatment of bacterial cells.

Statistical Analysis

Data obtained from at least three independent experiments were presented as mean ± SEM. The paired student t test (one-tailed) was used to determine significant difference between two groups of data. The p values of <0.05, <0.01 and <0.001 were considered as statistically significant and are indicated by asterisks (*, **, *** respectively). All the data were analyzed by using GraphPad 4.0 software

Results

Kirby-Bauer Assay

The morphology of C cresentus is depicted in figure 1. The antibiotic sensitivity assays were performed by treating Caulobacter cresentus with different dosages of the H_2O_2 such as 5mM, 10mM, 15mM, 20mM and different dosages of the bioactive compound. No zone formation was observed in all the concentrations of H_2O_2 (fig. 2 a) and the compound i.e selected doses were not lethal to the bacteria. Moreover, the selected doses of bioactive compound did not show anti-bacterial activity as well (fig. 2 b, c). H_2O_2 dose of 5mM was selected for further treatments (referred as H group). From the results of various treatments with higher to lower doses of R-L compound, 2 doses viz 2ng/ml (R-L1) and 100ng/ml (R-L2) was selected for further assays since these 2 doses caused the least harm to the bacterium.

Biochemical assays

Lipid peroxidase assay

The lipid peroxidation can be considered as marker for the detection of the oxidative stress. Lipid peroxidation was estimated in the cell lysate obtained from the treated bacteria. The accumulation of the free radical damage in the cell increases with the oxidative stress. The 5mM Hydrogen peroxide treatment induced more LPO activity when compared with the R-L1+H (2 ng/ml + 5mM H2O2) and R-L2+H (100 ng/ml + 5mM H2O2). The LPO activity is depicted in (fig. 3a)

Catalase assay

Catalase is the part of cellular defense against reactive oxygen species. In the non-senescent cells the catalase activity usually increases. The R-L1+H show enhanced catalase activity when compared to the hydrogen peroxide treated bacterial cell. The catalase activity is depicted in (fig. 3b) which displays beneficial role of the R-L compound.

Superoxide dismutase assay

The superoxide dismutase (SOD) is the first line of defense against ROS. The specific SOD activity of hydrogen peroxide treated group is more in the treated bacterial cell when compared with the bacterial cells treated with the bioactive compound. The SOD activity is seen to be less in R-L1+H and R-L2+H when compared with hydrogen peroxide. The superoxide dismutase activity is depicted in (fig. 3c)

Total thiol content assay

The amount of total thiol content is the major endogenous antioxidant produced by the cells. In the senescent cells the level of the GSH are usually low. The thiol content in the cell of hydrogen peroxide treated group is low when compared to the treated bacterial cell. The RL1+H and R-L2+H have more thiol content when compared with hydrogen peroxide. The total thiol content is depicted in (fig. 3d)

Anti-aging assays

Elastase Assay

The intracellular elastase increases in senescent cells due to the degradation of the elastin by the intracellular elastase. The inhibitory activity of elastase was observed in the control and the treated bacterial cells. The percentage inhibition of the enzyme elastase is low in the cells treated with the hydrogen peroxide. The percentage of inhibition is more in the R-L1+H when compared with the hydrogen peroxide. The elastase activity is depicted in (fig. 4a)

β-galactosidase assay

The beta-galactosidase is the commonly used markers for the cell-aging. The Caulobacter cresentus, was treated with R-L compound and hydrogen peroxide. The more activity of the beta galactosidase was observed in the hydrogen peroxide when compared with the bioactive treated compounds. The R-L2+H exhibited more activity when compared with the hydrogen peroxide. The graphis depicted in (fig. 4b)

Discussion:

The deterioration of the physiological functions leads to the process of aging and aging-related disorders. The safest alternative approach for delaying aging process in respect of orthodox therapeutic regime, is the usage of the naturally isolated bioactive compounds that have potentiality to delay the process of aging.[10, 11] Several natural compounds originating or derived from marine life including the actinomycetes, molluscs, cyanobacteria, ascidians, tunicates and sponges are now undergoing clinical trials .[12] Recently, Rhodiola Rosea extracts are used as a dietary supplement throughout Asia, Europe, and the United States enhancing physical and mental performance and fighting stress.[13] Our results depicted that the R-L compound (the bioactive fraction of Rhodiola which when combined with the lipoprotein fraction of Trachurus species was able to lessen the damage caused by hydrogen peroxide in bacterial species. The modulation in the anti-aging enzyme activity was also noted. These property might help to prolong the lifespan of the cells by maintaining the cellular integrity. The LPO is a free radical chain reaction mechanism that increases with the increase in metabolic process and age. In the LPO activity, the R-L compound exhibited effective suppression in free radical production. Catalase is the part of the cellular defense against reactive oxygen species (ROS) and catalase activity estimated that R-L1+H showed more catalase activity. Likewise, the superoxide dismutase (SOD) constitutes the first line of defense against ROS and as result shown, the lower dose of R-L compound increases SOD activity. The total thiol content assay was done to estimate the total thiol content in the bacterial cells treated with the bioactive compound which found to be reduced in R-L+H treated bacterial cell. It is the major endogenous antioxidant produced by the cells participating directly in the neutralizing of free radicals and reactive oxygen compounds. Moreover, in anti-aging assay, β- Galactosidase activity was observed to be lower than H_2O_2 treated group, which ensure reversal of cellular senescence by R-L compound and also more elastase enzyme inhibition activity observed in R-L treated bacteria cells. It is the supportive evidence for the ability of the bioactive compound to suppress the oxidative stress.[14-16] However, further extensive research is being carried in our laboratory on the combinational bioactive extract for testing the beneficial effects on various other immune modulating conditions.

Conclusion:

The R-L bioactive compound isolated from the natural sources acted to reduce damage caused by hydrogen peroxide-induced oxidative stress in bacteria cells. The bioactive compound usually possesses anti-oxidant and anti-aging properties that can prevent the cells from the damages caused due to intrinsic and extrinsic factors which was fairly evident by the results such as lipid peroxidase, catalase, superoxide dismutase and elastase. To

summarize, the overall results of the treatment displayed efficacy of natural compound Rhodiola rosea SBF and LF-Trachurus (R-L compound) against induced oxidative stress and anti-aging enzymes also depicted ameliorative results. The outcomes of this investigation might facilitate to target and to understand the therapeutic potential for marine natural products as promising sources of anti-oxidant and anti-aging compounds.

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