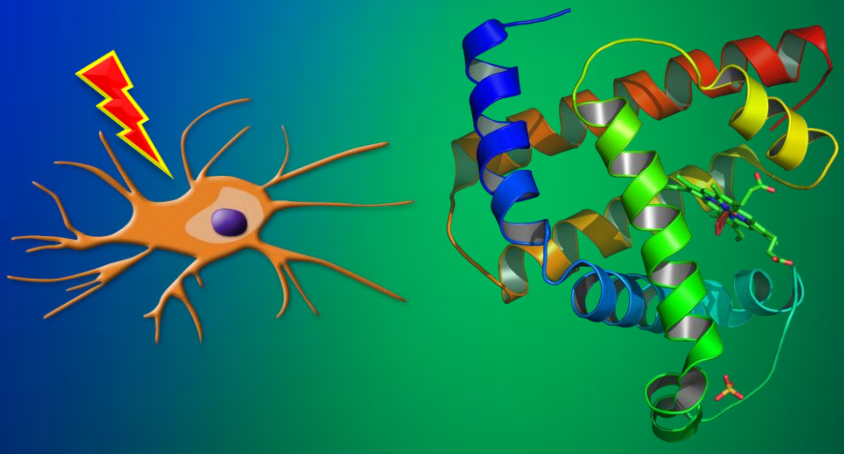
The Effect of Oxidative Stress on Protein Profile of Cells

Project Report



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ABSTRACT: Oxidative stress is associated with diseases such as Alzheimer's, heart failure, Parkinson's, Schizophrenia and others. Reactive oxygen species (ROS) is beneficial when used by our immune system to destroy pathogens. But severe ROS production (free radicals & peroxides) can cause cell death. Hydrogen peroxide (H_2O_2) is a potent inducer of oxidative stress in biological systems. Exposure to such a chemical results in alterations in synthesis of biomolecules by the cells including DNA and proteins. Antioxidants have been used to prevent disease but with limited success. There is a lack of effective drugs due to incomplete understanding of the stress response phenomenon. The present study aims at studying the variations in cellular protein profile in healthy and oxidatively damaged cells. Vitamin E (antioxidant) rescues cells from oxidative damage. Most interestingly, our findings suggest that oxidatively damaged cells are capable of producing some proteins that can protect normal cells from further damage due to oxidative stress caused by peroxides and free radicals.

BACKGROUND & RATIONALE: Cellular oxidative stress is generally defined by difference in reactive oxygen species (ROS) production and intracellular antioxidant molecule leading to potential damage (Halliwell & Gutteridge, 2007). In general, a high level of ROS production causes cellular damage. Any imbalance in the redox state of cells can cause toxic effects by production of free radicals resulting in complete cell damage, including DNA, proteins, and lipids. Protein expression in cells is required to maintain cellular homeostasis and survival. Abnormal and prolonged oxidant production may cause DNA damage leading to gene mutations (Visconti & Grieco, 2009). Oxidation of any protein can affect protein function leading to cell

death by apoptosis (Costa *et. al*., 2007). Overall protein production by the cells under stress might increase or decrease. Besides, some proteins usually minimally or not made by normal cells might be synthesized in response to the chemical- induced stress, and vice versa. Under disease conditions our body faces this challenge continuously. To be able to develop therapies that can reduce or eliminate the harmful consequences, it is necessary to understand the cellular stress response and associated functional changes.

HYPOTHESIS & OBJECTIVES:

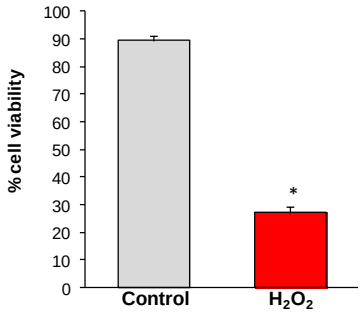
Oxidative stress causes changes in the protein expression profile of cells.

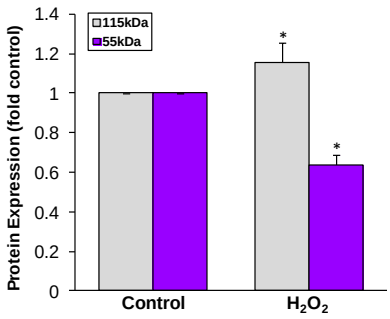
Specific objectives: 1) To investigate the effect of oxidative stress induced by H_2O_2 and UV radiation on cell viability and free radical generation. 2) To determine comparative protein expression profiles in healthy, stressed and antioxidant-treated cells.

PROCEDURE: A monolayer of Cos-7 cells (10^6 cells/ well) was treated with 100 µM of H_2O_2 for 24 hours in presence/absence of Vitamin E. Protein isolated from peroxide-treated cells was used for re-stimulation of healthy cells. UV radiation was also used to mimic induced-cancer properties. Cell-based assays included cell viability (Trypan blue exclusion test), ROS detection (CM-H_2DCFDA dye; Erslanov & Kusmartnev, 2010), and protein expression pattern analysis (gel electrophoresis). Data was recorded from three independent experiments (in triplicates), mean values were calculated and graphs were plotted in MS Office- Excel program.

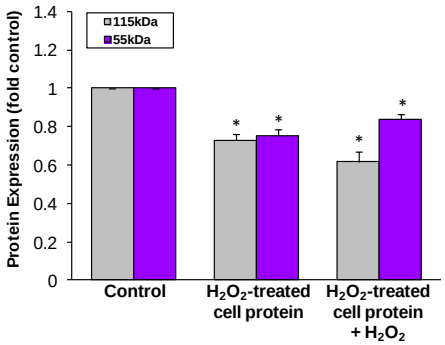
RESULTS:

1. In response to induction of oxidative stress by H_2O_2, cell viability decreases significantly; ROS production increases; protein expression is altered (⇧115kDa, ⇩55kDa).

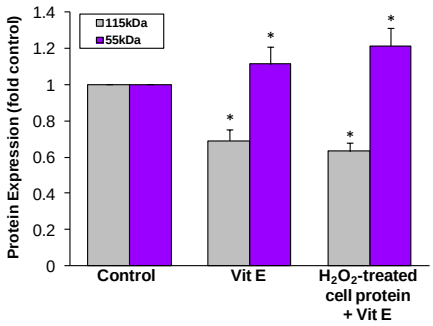




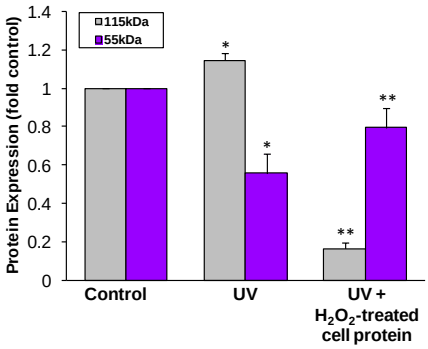
2. On exposing healthy cells to protein from H_2O_2-treated cells, overall protein expression is lowered (⇩115kDa, ⇩55kDa). When these cells are further exposed to peroxide, cell viability decreases; ROS production slightly increases; protein expression pattern is reverse of H_2O_2 group (⇩115kDa, ⇧55kDa).



3. Vitamin E treatment helps maintain cell viability; ROS production is negligible; protein expression is lowered (⇩115kDa, ⇩55kDa) under normal conditions. In cells stimulated with protein from H_2O_2-treated cells and Vitamin E exposure, protein expression pattern is similar to Vitamin E group. Interestingly, the expression of 115kDa protein is lower and 55kDa protein is comparatively higher than Vitamin E group.



4. In UV- irradiated cells, cell viability ⇩; ROS⇧; protein expression similar to H_2O_2 group indicating stress response. Exposure to protein from H_2O_2-treated cells reverses the effect suggesting the protective role of this protein as an antioxidant.



SIGNIFICANCE: Protein profiling is a precise and effective method for monitoring protein synthesis in cells under healthy as well as disease conditions. Targeting the high (115kDa) and low (55kDa) molecular weight proteins from our study may be beneficial in protecting cells from oxidative damage. Our future experiments will elucidate the functional role of these proteins in cells and tissues and provide basis for a potential target for therapeutic development.

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