The Effect of Differential Molecular Weight Protein in Rescue of Oxidative Damage

Project Report

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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:

"Proteins synthesized by oxidatively damaged cells have variable effects on cell health."

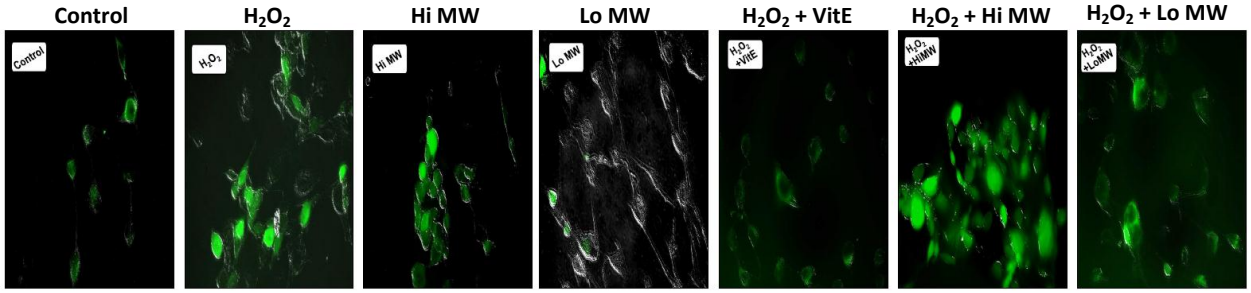
Specific objectives: **1)** To investigate the effect of differential molecular weight proteins on ROS generation and cell health under healthy and oxidative damage conditions, **2)** To determine the affinity of normal and damaged cells towards the high MW and low MW proteins using a chemotaxis assay.

PROCEDURE: A monolayer of Cos-7 cells (10^6 cells/ well) was treated with 100 µM of H_2O_2 for 24 hours. Total protein isolated and purified partially using column filters to separate protein higher (Hi-MW) and lower (Lo-MW) 100kDa molecular weight. Purified proteins were confirmed by SDS-PAGE. Cos7 cells were exposed to 100 µM of H_2O_2 for 24 h in presence of Hi-MW or Lo-MW protein fractions. Cell-based assays included cell viability, cytotoxicity and apoptosis (ApoTox-Glo™ Triplex Assay), ROS detection (CM-H_2DCFDA dye; Erslanov & Kusmartnev, 2010), protein expression analysis for pro-apoptotic Bax & anti-apoptotic Bcl_{XL} proteins (SDS-PAGE followed by western blotting), and Chemotaxis assay (Mousseau *et. al.*, 2007). Data was recorded from three independent experiments (in triplicates), mean values were calculated and graphs were plotted in MS Office- Excel program.

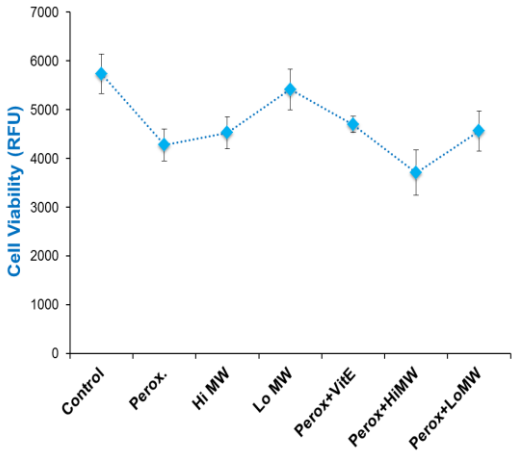
RESULTS:

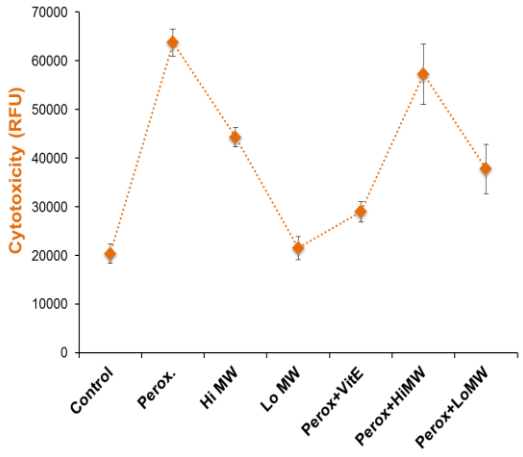
1. **Partial purification of H_2O_2-treated cell protein:** A high molecular protein (HiMW:~115kDa) and a low molecular weight protein (LoMW:~55kDa) were purified using column filters and confirmed by SDS-PAGE.

2. **ROS Production:** ROS production increased in response to addition of Hi MW whereas Lo MW fraction had an opposite effect. Furthermore, when used in combination with peroxide, Hi MW caused a signification increase in ROS production whereas Lo MW partially inhibited this surge.

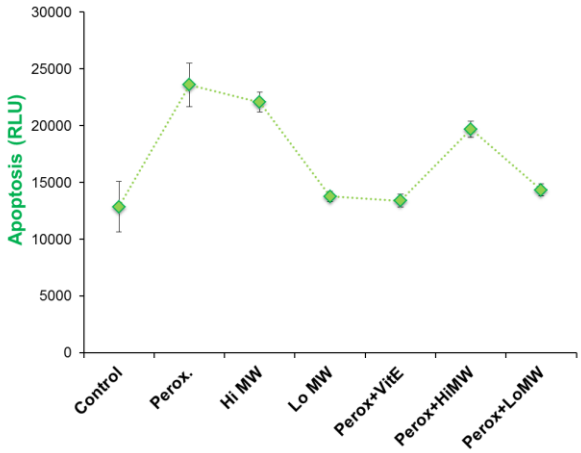


3. **Cell Viability & Cytotoxicity:** Cell viability plot suggests that Hi MW protein is detrimental to cell survival. This protein is also highly cytotoxic compared to its Lo MW counterpart.

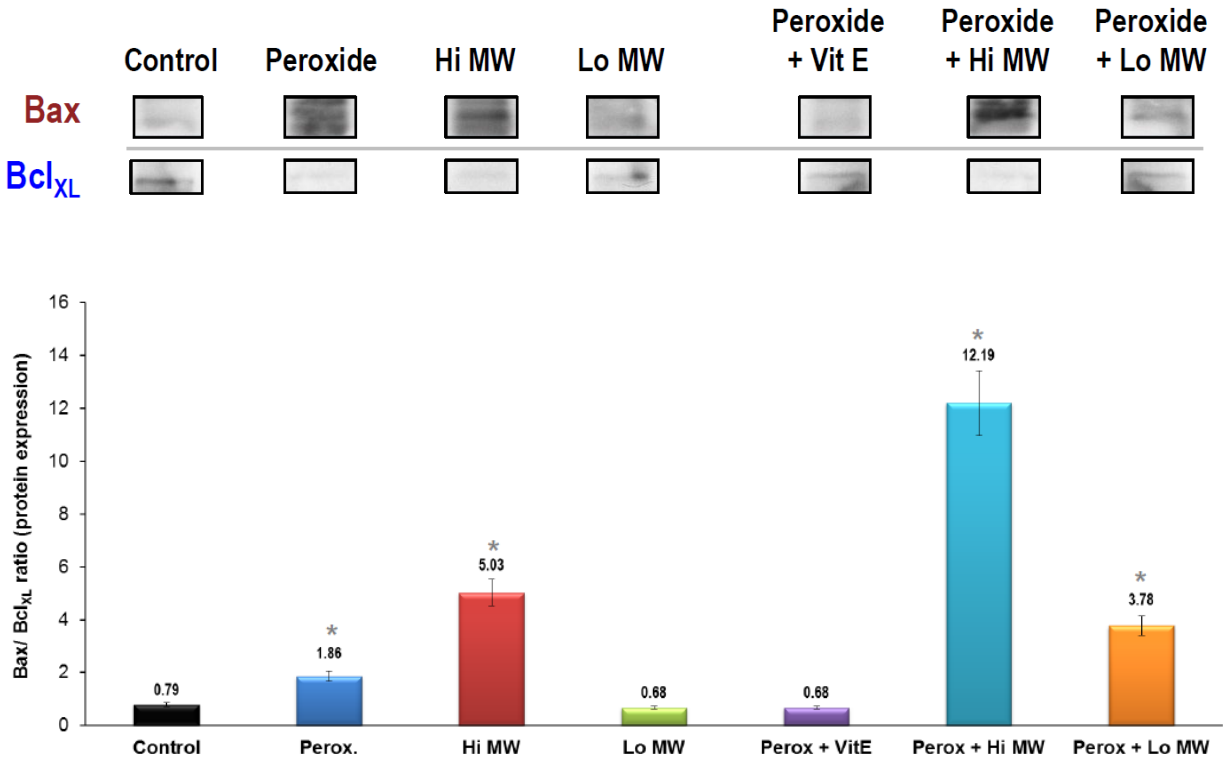




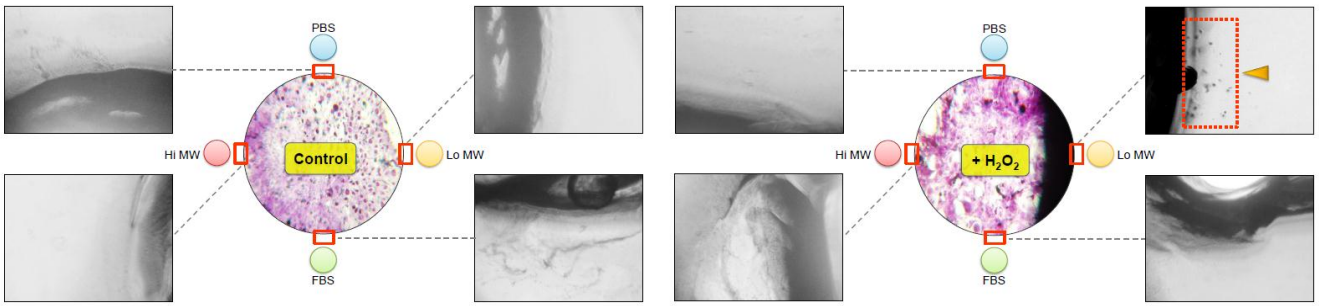
4. **Apoptosis OR Caspase3 activity:** The Hi MW fraction triggers apoptosis to an extent similar to peroxide, whereas the Lo MW protein appears to have similar anti- apoptotic properties as Vitamin E.



5. **Pro-apoptotic Bax and Anti-apoptotic Bcl-xL protein expression:** Pro-apoptotic protein Bax is highly expressed in cells exposed to Hi MW protein compared to Lo MW protein. The reverse is true for anti-apoptotic protein Bcl_{XL}.



6. **Chemotaxis assay:** A chemotaxis assay showed that damaged cells have strong affinity towards the Lo MW protein suggesting its potential protective property compared to its Hi MW counterpart. Under healthy conditions, there is no preference for either possibly due to a balanced synthesis between them.



CONCLUSIONS:

1. ROS production increases in response to addition of Hi MW whereas Lo MW fraction has an opposite effect. When used in combination with peroxide, Hi MW causes a signification increase in ROS production whereas Lo MW partially inhibits this surge.
2. Hi MW protein is detrimental to cell survival. This protein is also highly cytotoxic compared to its Lo MW counterpart. The Hi MW fraction triggers apoptosis to an extent similar to peroxide, whereas the Lo MW protein appears to have similar anti- apoptotic properties as Vitamin E. Pro-apoptotic protein Bax is highly expressed in cells exposed to Hi MW protein compared to Lo MW protein. The reverse is true for anti-apoptotic protein Bcl_{XL}.
3. Damaged cells have higher affinity towards the Lo MW protein suggesting its potential protective property compared to its Hi MW counterpart.

SIGNIFICANCE: The lack of a perfect therapy for oxidative damage is in part due to an incomplete understanding of the process. Our present findings suggest that modulating cell protein synthesis to produce rescue proteins more than damage- causing ones may hold the key to a sustainable therapy for the problem. Future studies will help elucidate their functional role in cells and tissues and provide basis for a potential target for therapeutic development.

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