Melanin relieves oxidative stress in adipocytes culture in vitro

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ABSTRACT

Obesity is one of major health disorders that has become epidemic worldwide. Studies have demonstrated that oxidative stress and inflammation in adipose tissue is involved in obesity linked complications. The ectopic synthesis of melanin in adipocytes of obese patients has been newly discovered. It is hypothesized that there is potential role of melanin and its intermediates to ameliorate oxidative stress in adipose which may prevent the secondary complications to obesity. This study was done to determine whether melanin interferes with the level of oxidative stress produced in adipocyte cells in culture. Adipocyte cells were cultured in vitro and exposed to high concentration of glucose oxidase to induce oxidative stress, then cells were treated with melanin for different periods. Thereafter the levels of some oxidative stress and inflammatory biomarkers such as glutathione, TNF-alpha, Malondialdehyde (MDA), and protein carbonyl were measured. It was found that when cells were exposed to glucose oxidase the level of oxidative stress increased over time as reflected by the decrease of Glutathione. Interestingly, when melanin was added the level of oxidative stress decreased over time as reflected by increase in glutathione concentrations. It was also found that TNF-alpha decreased upon treatment by melanin but after 72 hours but not on 24 and 48 hours treatments. However, protein carbonyl and MDA appeared to be less affected by melanin treatments. These results may indicate that melanin is capable of diminishing the effects of oxidative stress on the cells at early stages probably by interfering with lipids peroxidation and production of reactive oxygen species.

Keywords: Oxidative stress, Melanin, Adipocytes, Obesity, Biomarkers

Introduction

Oxidative stress is an imbalance between pro-oxidants and antioxidants in the body and evident in many disease states including obesity. Obesity is a disease responsible for many complications including insulin resistance, cardiovascular disease and NAFLD (nonalcoholic fatty liver disease). The severity of the complications differs in obese patients and not correlated with obesity level. These complications are due to expanded adipose tissue releasing high levels of pro-inflammatory. In addition, reactive oxygen species (ROS) have
been shown to be increased in obesity and implicated in many consequent complications.\cite{3,4} Hydrogen peroxide is known to be an oxidizing agent as well as a ROS. Therefore, the increase or buildup of hydrogen peroxide due to high glucose oxidase has shown to cause an increase on the rate of oxidative stress in the mature adipocyte cells.\cite{5}

Melanin synthesis and the melanogenic pathway components have been recently found to be active in different levels in adipose of obese subjects.\cite{6,7} It has been proposed that the melanin synthesized in abundance in obese individuals could have role in abating obesity-related complications through its well-known antioxidant and free radicals scavenging activities.\cite{8}

In this short research study, our objective was to test if melanin is capable of relieving the glucose oxidase induced oxidative stressed adipocytes in vitro culture by slowing down or stopping the action of ROS produced from lipid peroxidation in adipocyte cells.\cite{7,9}

**Materials and Methods**

Matured human subcutaneous adipocyte cells in 24 wells microplate were purchased from ZenBio and upon arrival were left in a CO\textsubscript{2} incubator until the next day. They were observed under microscope and the cells were healthy looking. The matured adipocyte cells were treated with 50 mU/ml glucose oxidase to induce oxidative stress, thereafter treating the cells with 100 µM melanin for 24, 48 and 72 hours. Then we measured the levels of some of the oxidative stress biomarkers such as MDA, Glutathione, TNF-alpha and protein carbonyl, according to procedure manuals; in the cells before and after treatment with melanin.

In brief description, the 24 well-plate each well containing 80,000 cells was divided into 3 sets. One set was the adipocyte cells which were treated with glucose oxidase only and were harvested at 24 hour intervals as positive controls. Second set of cells were left without glucose oxidase treatment and extracted at 24 hour intervals as negative controls. The third set was for cells with glucose oxidase treated with melanin (100 µM) collected at 24 hour intervals.

Glutathione levels were estimated by using the QuantiChrom™ Glutathione Assay by BioAssay Systems, Melondialdehyde (MDA) measurements were carried out using the OxiSelect™ TBARS Assay Kit, Protein Carbonyl content was assessed using the OxiSelect™ protein carbonyl ELISA kit by Cell Biolabs, and TNF-alpha level was determined by human ELISA Kit by Abcam.

**Results and Discussion**

The new adipocyte cells upon arrival were checked under microscope and appeared to be healthy. Then after treatments the cells were observed under microscope daily to monitor their health and any changes. Cells exposed to Glucose Oxidase (100 mU/ml) at 24 hour incubation intervals compared to cells with no treatment showed reduction in glutathione concentration as the exposure time increased (Figure 1A). Control cells without treatment and cells exposed to glucose oxidase for 24 hours exhibited a glutathione concentration of 3 µM and at 72 hours the concentration decreased by 50%. The decline in glutathione concentration related to time of exposure is related to oxidative stress.

The concentration of glutathione (µM) then was measured in cells that were treated with both glucose oxidase and melanin (100 µM). It shows that when melanin was added to the cells with glucose oxidase there is an increase in glutathione concentration after 48 hours and 72 hours of treatment (Figure 1B). However, after only 24 hours of exposure the concentration of glutathione decreased by 34%. The improvements in glutathione concentrations in adipocytes are clearly shown due to the melanin antioxidative stress capability and this effect may be time specific.

We then tested for the concentration of the MDA (µM) in cells treated with glucose oxidase (50 mU/ml) and melanin (100 µM) with 24 hour intervals of exposure. The cells treated only with glucose oxidase were tested against cells with
glucose oxidase and melanin. It is found that there is almost no variations in the concentration of MDA regardless of the different incubation times with the 100 \( \mu \text{M} \) concentration of melanin (Figure 1C). Also Treatment of cells with melanin showed almost no effect on protein carbonyl compared to cells treated with glucose oxidase only.

The concentration of TNF-alpha was tested in cells exposed to glucose oxidase only and in cells with glucose oxidase but also treated with melanin exposed for intervals of 24 hours. Figure 1D shows an increase and then a sudden decrease in the concentration of TNF-alpha as time progresses. The figure also shows that cells with glucose oxidase treated with melanin exposed for 72 hours has the lowest concentration of TNF-alpha, lower than the concentration in the cells with glucose oxidase only.

From our results provide preliminary support that melanin could have anti-oxidative stress in adipose. As we notice that early oxidative stress markers as glutathione and TNF-alpha are more directly to be influenced with melanin treatments than late biomarkers as MDA and protein carboxylation. Melanin anti-oxidative stress capabilities are more limited to ameliorating the ROS resulted from lipids peroxidation or may it would need longer time for melanin to have anti-MDA, HNE or protein carboxylation in oxidative stressed adipocytes. Overall this needs more research.

Our results can be considered as encouraging preliminary foundation for more comprehensive future research. In addition to melanin, we recommend to use one of the soluble intermediates of melanin, DHICA as it is water soluble and can naturally diffuse into adipose tissue reflecting more the \textit{in vivo} nature of its possible anti-oxidative role in adipose.
Experimenting with different concentrations of Glucose oxidase and Melanin and DHICA for longer time exposures may also be an alternative for obtaining more comprehensive supporting results. Moreover, testing other biomarkers and studying the different gene expression of each biomarker may provide further understanding as well.

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