ASCORBIC ACID ATTENUATES HYDROGEN PEROXIDE INDUCED OXIDATIVE STRESS AND OSTEOBLASTS DEMONSTRATE ANTIOXIDANT RECYCLING POTENTIAL

Richard T. Tran, Joshua L. Morgenstern, Steve Droho, Jeffrey L. Olson

INTRODUCTION

Oxidative stress is strongly implicated in the disease progression of age-related macular degeneration. Oral supplements, including ascorbic acid, target this oxidative etiology, yet their efficacy is limited due to insufficient ocular distribution. One possible avenue is restoring the antioxidant potential of the vitreous by improving recycling of the inactive oxidized form of ascorbic acid (AA), dehydroascorbic acid (DHA), back to its active reduced form.

Here, we evaluate the ability of osteoblasts to act as a source of antioxidant recycling. Additionally, we demonstrate the antioxidant potential of ascorbic acid to improve common retinal pigment epithelium (ARPE-19) cell viability in the setting of H$_2$O$_2$ induced oxidative stress.

METHODS

Antioxidant Recycling:

Osteoblast antioxidant recycling potential was evaluated in vitro by exposing MG-63 osteosarcoma cells to culture containing DHA. Cells were grown in media recommended by ATCC and allowed to proliferate until 80% confluence to ensure high metabolic activity. Cells were subsequently washed in sterile PBS to remove residual media and were exposed to media with and without 100uM DHA. At each time point from 0 to 80 minutes, media was collected and concentrations of DHA and ascorbic acid were assessed using HPLC. Statistical comparisons were performed using a student’s t-test.

RESULTS

Cell Viability:

Ascorbic acid successfully attenuated the toxic effects of H$_2$O$_2$, with 88% of ARPE-19 cells remaining viable after exposure to both H$_2$O$_2$ and ascorbic acid, compared to 61% viable after incubation with 0.3mM H$_2$O$_2$ alone (P < 0.001***). When exposed to 0.4mM H$_2$O$_2$, both groups showed markedly reduced viability, suggesting oxidative species overwhelming the protective effects of ascorbic acid.

Antioxidant Recycling:

Osteoblast antioxidant recycling of DHA was observed through an increase of ascorbic acid concentration over time in DHA media compared to control media. At 80 minutes, the concentration of ascorbic acid had a 2-fold increase. Concomitantly, DHA levels decreased over time, supporting the regeneration of ascorbic acid from DHA by osteoblasts.

DISCUSSIONS

None

ACKNOWLEDGEMENTS

This work was conducted in the Olson Lab with funding from the University of Colorado School of Medicine Research Track.

REFERENCES


Richard T. Tran, Richard.T.Tran@cuanschutz.edu