

## Title

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

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## Purpose of Study

Oxidative stress is strongly implicated in disease progression of age-related macular degeneration. Oral supplements, including ascorbic acid (AA), target this oxidative etiology, yet 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 AA, dehydroascorbic acid (DHA), back to its active reduced form. Here, we demonstrate the antioxidant potential of AA to improve common retinal pigment epithelium (ARPE-19) cell viability in the setting of  $H_2O_2$  induced oxidative stress and evaluate osteoblasts as a potential source of antioxidant recycling.

## Methods Used

In vitro evaluation was performed by incubating ARPE-19 in culture media containing .2mM  $H_2O_2$  with and without 100uM AA. MTT assay was performed to assess for cell viability. Osteoblast antioxidant recycling potential was tested by exposing MG-63 osteosarcoma cells to culture media containing 100uM DHA. At each time point from 0 to 80 minutes, media was collected and concentrations of DHA and AA were assessed using HPLC. Statistical comparisons were performed using a student's t-test.

## Summary of Results

AA successfully attenuated the toxic effects of  $H_2O_2$ , with 88% of ARPE-19 cells remaining viable after exposure to both  $H_2O_2$  and AA, compared to 61% viable after incubation with  $H_2O_2$  alone ( $P < .001$ ). Osteoblast antioxidant recycling of DHA was observed with an increase of AA concentration and a concomitant decrease in DHA levels over time. At 80 minutes, the concentration of AA had a 2-fold increase with a paired 2-fold decrease in DHA levels.

## Conclusions

These experiments demonstrate the antioxidant potential of AA to attenuate the effects of oxidative stress and its physiologic importance in managing cellular exposure to reactive oxygen species. Osteoblasts exhibited the potential for antioxidant regeneration of AA outside their biological niche. While preliminary, these results demonstrate the promise of an implantable device that continuously recycles antioxidant, eliminating the need for constant injections.