Ascorbic Acid Attenuates Hydrogen Peroxide Induced Oxidative Stress and Osteoblasts Demonstrate Antioxidant Recycling Potential

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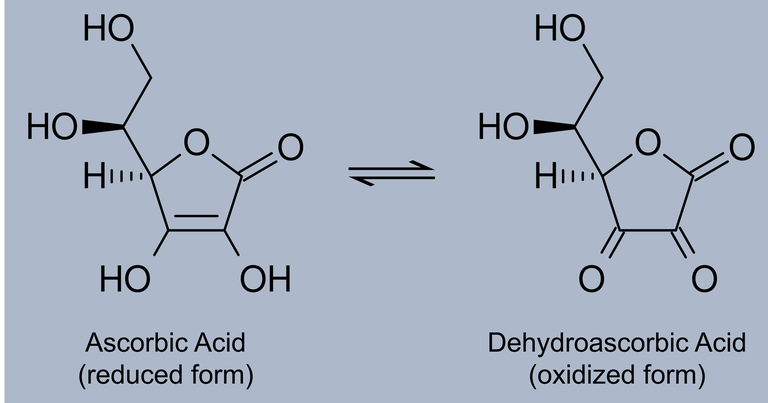
INTRODUCTION

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, 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_2O_2 induced oxidative stress.



Ascorbic Acid (reduced form) | Dehydroascorbic Acid (oxidized form)

Figure 1: Oxidation/reduction schematic of AA and DHA
Ascorbic acid (AA) absorbs oxidative damage by acting as a reducing agent and converting to dehydroascorbic acid (DHA). To restore the reducing potential of AA, its oxidized form DHA must be converted back to AA.

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.

Cell Viability:
Ascorbic acid antioxidant protective effects were evaluated in vitro by measuring survival of cells exposed to H202.

METHODS

ARPE-19 cells were cultured, with one group given 100uM ascorbic acid media and the other group given control media. The groups were incubated in 0.2mM and 0.4mM H202 overnight, and MTT assay was performed to assess for cell viability.

RESULTS

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.

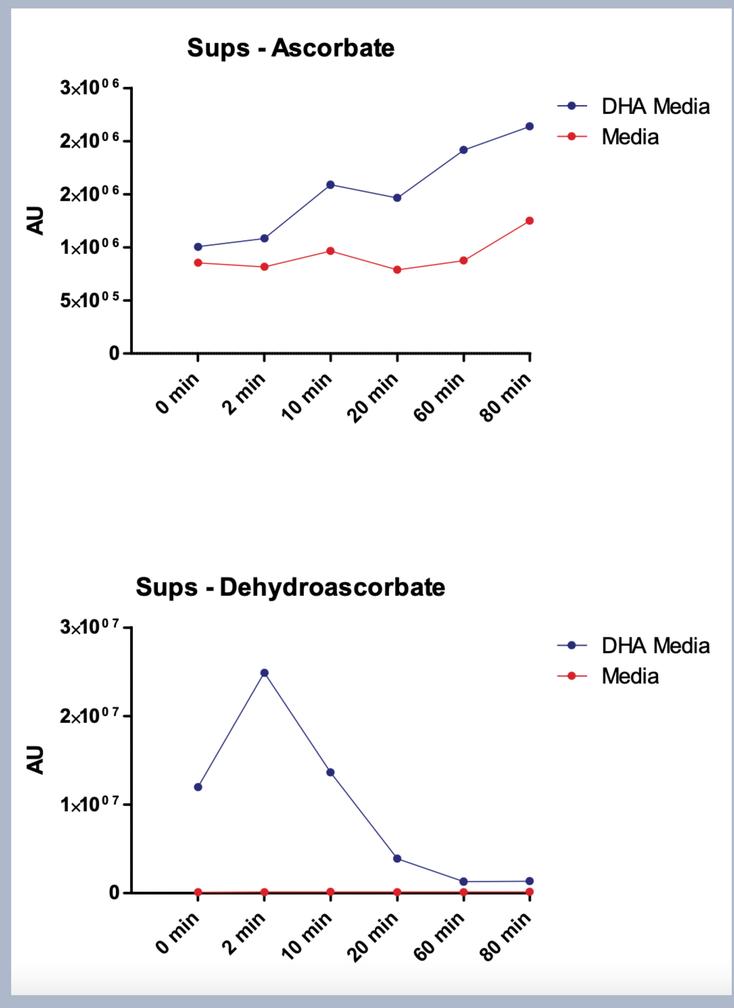


Figure 2: Osteoblast Recycling of DHA to AA
Ascorbic acid levels (top graph) were shown to increase while DHA levels (bottom graph) were shown to simultaneously decrease, both as compared to control.

RESULTS

Cell Viability:
Ascorbic acid 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 ascorbic acid, compared to 61% viable after incubation with 0.2mM H_2O_2 alone ($P < 0.001$***). When exposed to 0.4mM H_2O_2, both groups showed markedly reduced viability, suggesting oxidative species overwhelming the protective effects of ascorbic acid.

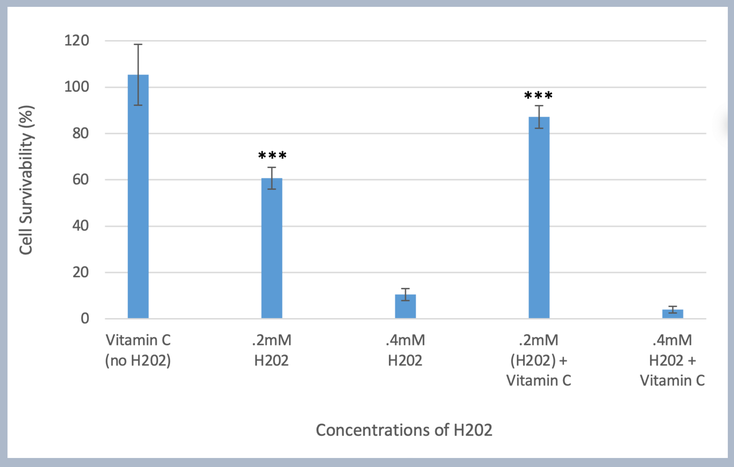


Figure 3: Ascorbic Acid Protects ARPE-19 Cells From Oxidative Stress-induced Cell Death.
When exposed to 0.2mM H_2O_2, ARPE-19 cells incubated with ascorbic acid demonstrated greater viability than cells without ascorbic acid, with 88% survivability compared to 61%, respectively ($P < 0.001$***).

DISCUSSION

In these experiments, osteoblasts demonstrated the potential for regeneration of ascorbic acid outside the cells' biological niche. Theses experiments also exhibit the antioxidant potential of ascorbic acid to attenuate the effects of oxidative stress and its physiologic importance in managing cellular exposure to reactive oxygen species.

While preliminary, these results demonstrate the promise of an implantable device that continuously recycles antioxidant, eliminating the need for constant injections. Further work includes development of such an intraocular device to treat age-related macular degeneration and diseases with similar oxidative pathophysiology.

ACKNOWLEDGEMENTS

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