Pharmacological Ascorbic Acid and Hyperbaric Oxygen Therapy Target Tumor Cell Metabolism via an Oxidative Stress Mechanism

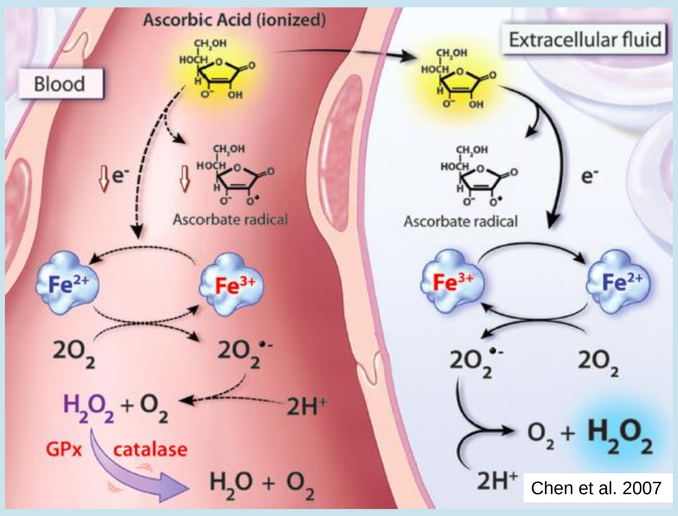
Janine M. DeBlasi, Nathan P. Ward, Angela M. Poff, Andrew P. Koutnik, Christopher Q. Rogers, David M. Diamond, Dominic P. D'Agostino

Department of Molecular Pharmacology and Physiology, University of South Florida, Tampa, FL

Hyperbaric Biomedical Research Laboratory
supported in part by:
DEPARTMENT OF THE NAVY
ONR
Science & Technology

Background Information

- Cancer is the second leading cause of death in the U.S.
  - Projected to take 595,690 lives in 2016 and cost the nation over $125 billion
- To effectively reduce these detrimental losses, **non-toxic, low-cost therapies** should be further examined to supplement the standard of care
- Anti-carcinogenic and minimally toxic therapy under investigation: **high-dose ascorbic acid (AA)**
- High-dose AA has elicited significant **anticancer effects** in animal models and small-scale human reports at concentrations **nontoxic** to healthy cells
- AA can function as a **pro-oxidant** at pharmacological levels (achieved I.V. or I.P.)
  - Delivers H_2O_2 to tumorous tissue upon oxidation and selectively initiates cell death in cancer cells



Aims of Experiment

- We aim to examine the **anticancer effect** of AA *in vitro*, to mechanistically evaluate AA-induced **oxidative stress,** and to investigate AA's **synergy** with another non-toxic, pro-oxidative metabolic therapy: **Hyperbaric Oxygen (HBOT)**
  I. Determine the effect of AA on viability and proliferation *in vitro*
  II. Evaluate the mechanism of AA-induced cytotoxicity: N-Acetyl cysteine (NAC) is an antioxidant precursor to glutathione, an antioxidant that is highly abundant in the body and scavenges free radicals. If treatment with NAC attenuates the therapeutic effect of AA, this finding would support the hypothesis that **oxidative stress mediates AA-induced cytotoxicity**
  III. Investigate if synergy exists between HBOT and AA: HBOT is a medical treatment used to heal wounds, radiation injury, decompression sickness, and other health ailments by delivering 100% oxygen at elevated barometric pressure, elevating tissue pO_2 and oxygenating hypoxic tumor cells, which, when coupled with high levels of reactive oxygen and nitrogen species present in cancer cells, can further augment **oxidative stress** and lead to cell death. Thus, we hypothesize that HBOT will synergize with AA and further decrease VM-M3 cell viability
- We anticipate that this approach will yield significant insight and further investigate into the hypothesis that AA and HBOT can serve as **adjuvant therapies** to the standard of care

Experimental Design

VM-M3 Cells:
- Highly metastatic cells derived from a spontaneous brain tumor in a VM/Dk inbred mouse

AA-induced cell death:
- Cytotoxicity/ viability was measured in VM-M3 cells with fluorescence microscopy using dyes calcein AM and EthD-1 to identify live and dead cells, respectively. Cells labeled with both calcein AM and Ethd-1 may indicate early stages of necrosis and were counted as dead (Ethd-1 binds with nucleic acids inside the cell, indicating a loss of membrane integrity)
- Cells were treated with pharmacological concentrations of AA 0.001- 5mM

AA's effects on proliferation:
- Standard trypan blue hemocytometry measured proliferation
- Cells were treated with graded concentrations of AA and were counted after 24, 48, 72, and 96 hours of growth

Treatment with antioxidant NAC and AA:
- Cells were treated with a cytotoxic concentration of AA (0.5mM) in the presence or absence of 0.5 and 5mM NAC

AA and HBOT Combination:
- Cells were treated with one session of HBOT (100% O_2, 60 mins, 2.5 ATA)
- AA concentrations <0.5mM were used since ≥0.5mM AA are already cytotoxic

Ascorbic Acid & HBOT inhibit VM-M3 Cells via Oxidative Stress

VM-M3 Cell Death and Proliferation Inhibition

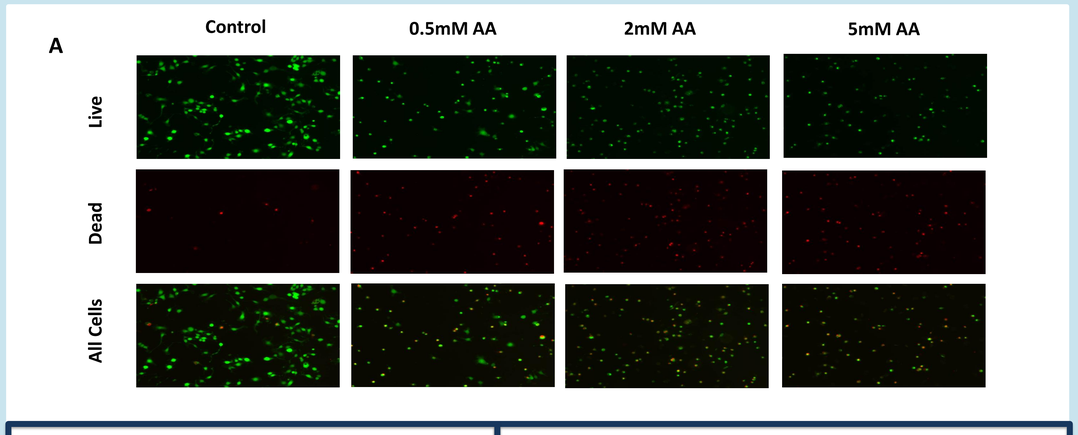


Figure 1. AA mediates VM-M3 cell death in a concentration-dependent manner. (A,B) 24 hour treatment with 0.5, 2, and 5mM AA significantly induced cytotoxicity compared to control and AA concentrations ≤0.3mM (One-way ANOVA, ****p<0.0001).

Figure 2. AA decreases VM-M3 cell proliferation *in vitro*. 0.05, 0.1, 0.3, and 0.5mM AA significantly decreased VM-M3 cell proliferation compared to control and 0.01mM AA at 72 and 96 hours of growth (Two-way ANOVA, p<0.05). 0.1, 0.3, and 0.5mM AA were also significant in comparison to 0.05mM AA at 96 hours of growth (Two-way ANOVA, p<0.05).

Effect of NAC on AA-mediated Cytotoxicity

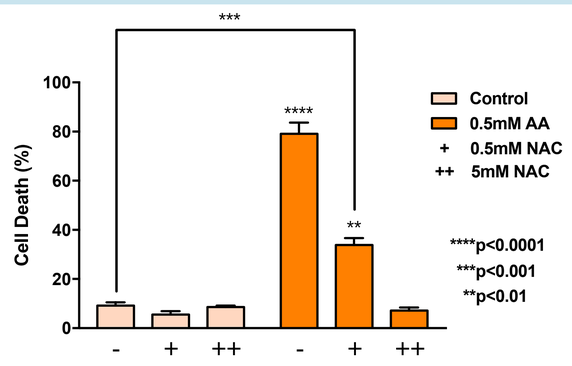


Figure 3. Antioxidant NAC attenuates the effect of AA *in vitro*. 24 hour treatment with 0.5 and 5mM NAC mitigated AA-induced cytotoxicity (One-way ANOVA, ****p<0.0001).

Potential Synergy of AA and HBOT

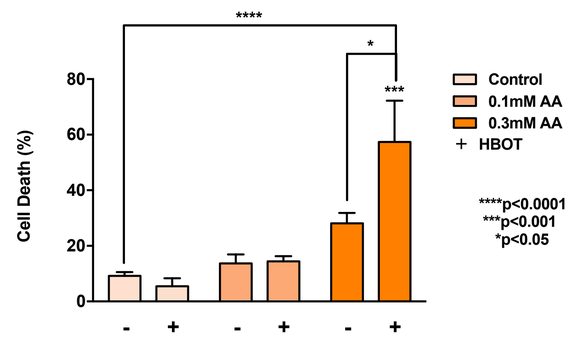


Figure 4. HBOT and AA synergize *in vitro*. 24 hour treatment with HBOT and 0.3mM AA significantly increased cytotoxicity compared to all other treatments (One-way ANOVA, p<0.05). The addition of HBOT did not affect control and 0.1mM AA.

Conclusions

- Pharmacological AA shows an **anticancer effect** *in vitro* and exhibits cytotoxicity through an **oxidative stress** mechanism that is therapeutically exploited by **HBOT**
- Our findings indicate that these non-toxic, pro-oxidative metabolic therapies should be further investigated as **adjuvants** to the current standard of care
- Further studies include:
  - Evaluating the effect of HBOT on the proliferation of AA-treated VM-M3 cells
  - Evaluating role of hydrogen peroxide (H_2O_2) in AA-induced cytotoxicity with treatment of catalase, an enzyme that breaks down H_2O_2 to H_2O and O_2
  - Investigating AA's selective cytotoxic mechanism
  - Examining AA and HBOT in combination with radiation therapy

Acknowledgements

Facilities use at Laboratory of Metabolic Medicine (Director, Dr. Dominic D'Agostino) and Hyperbaric Biomedical Research Laboratory (Director, Dr. Jay B. Dean). Work supported by: USF Foundation (501c3); Metabolic Therapy and Cancer Research Account (#250244): Scivation: Florida High Tech Corridor Funding (#MED052-0061361)