THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DIVISION OF ARTS AND HUMANITIES

# ANTIPROLIFERATIVE EFFECT OF ASCORBATE ON BREAST EPITHELIAL CELLS ACTS VIA A PRO-OXIDANT MECHANISM OF HYDROGEN PEROXIDE PRODUCTION

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## ABSTRACT

Ascorbate, otherwise known as vitamin C, has been well known for its use in supplementation against the common cold, stimulating the immune system, but work originating with Linus Pauling suggests it may have anti-cancer properties. Ascorbate has a variety of proposed mechanism including: boosting immunological activity, cancer protein degradation, glycolytic enzyme inhibition, and reactive oxygen species scavenger depletion. One of the main supported mechanisms is that of hydrogen peroxide production to perturb redox homeostasis within cancer cells that cannot cope to the imposed redox stress. Cancer cells often lack enzymatic activity to control free radicals and hydrogen peroxide. *In vitro* culturing involved a variety of breast epithelial cell lines cultured in high-calcium medium and incubated for three days. Results suggest that ascorbate decreases cellular proliferation in a dose-dependent manner. The tumorigenic T4 cells warrant the use of a higher concentration of ascorbate possibly up to 30-mM, which has been found to be achievable *in vivo.* Catalase experimentation was used to target hydrogen peroxide; cell lines treated with both, ascorbate and catalase, exhibited a normalized growth resembling the control 0-mM flasks. Our negative results suggest ascorbate acts via a pro-oxidant mechanism to decrease cellular proliferation. High-dose ascorbate treatment as an adjuvant to cancer treatment offers a great method to further decrease cancer proliferation as shown in these *in vitro* studies.

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# Chapter 1

## Introduction

Cancer is a disease that many know to be associated with abnormal cell growth, resulting in tumors, often leading to various complications. Recently, it has been found that ascorbate, otherwise known as vitamin C, has been found to slow the rate of growth of particular cancer cells and tumors including, but likely not limited to: prostate, pancreatic, hepatocellular, colon, and neuroblastoma (2, 5, 9, 10). Ascorbate is believed to have many mechanisms attributed to its activity, other than the well-known immunological boosting activity (6). Many mechanisms have been proposed to cause this anti-proliferative effect, and they are often interconnected. The mechanism of interest in my experimentation was that of a pro-oxidant. Ascorbate acts to produce free radicals that decrease the proliferation of cancer cells; the particular free radical of interest in this study is hydrogen peroxide. Hydrogen peroxide acts as an excellent oxidizing agent that can alter the activity of many enzymes and increase the reactivity, as well as the instability, of various molecules.

Due to ascorbate's structural resemblance to glucose, GLUT1 and GLUT3 transporters have been found to transport ascorbate into the cancer cells (12, 13, 16). In cancer cells modified by *KRAS* and *BRAF* mutations, the transport of dehydroascorbate into the cells was greatly increased (17). Other studies suggest the importance of extracellular hydrogen peroxide production, but this provides an intracellular mechanism likely related to glycolytic enzymes.

Hydrogen peroxide is often decomposed in healthy cells to prevent superoxide damage by the action of the enzyme catalase. Catalase has the strongest specificity to target hydrogen

peroxide free radicals for neutralization. Cancer cells often experience lower levels of oxidative stress because of the dependence on glycolysis for energy production rather than oxidative phosphorylation through the electron transport chain. Exposure to oxidative stress due to ascorbate may be enough to disrupt the cellular balance within the cancer cells. Ascorbate treatment has high value due to its propensity to not damage normal cells; no damage to normal cells may be attributed to the ability of normal cells to handle oxidative stress by expressing catalase as well as other reactive oxygen species scavengers.

Current chemotherapeutic drugs target all cells, not just the cancer cells, further precipitating issues for patients and possibly putting them at risk for cancer's recurrence. Ascorbate treatment would largely target only cancerous tissues rather than healthy, non-deleterious tissue as found in phase I clinical trials as well as *in vitro* studies (8, 10). Deviating from nonmalignant cellular death may assist in a patient's quality of life and may improve the effectiveness of the chemotherapeutic drugs via further suppressing cancer growth.

## Biological History

Ascorbate not only carries interest in biological aspects, but historical as well. Scientist, Linus Pauling, was the first to suggest the curative potential of ascorbate on a myriad of conditions. In Pauling's study on ascorbate, he found results including: decreased size of tumors of pancreatic origin; a reported greater quality of life; and possibly decreased cancer metastasis (2). The Mayo Clinic examined Pauling's research, following experimental repetition of his study, they found no significant effect of ascorbate. Mayo Clinic's results put ascorbate research on hold for nearly twenty years. The Mayo Clinic method of administration is the key to their

results, rather than intravenous administration of the ascorbate, they relied purely on oral doses; Pauling had an intravenous administration as well as an oral dosage. The gastrointestinal absorption of ascorbate is limited by a variety of factors including: molar concentration in the small intestine, concentration within the blood, and osmolarity of the intestinal tissues (12). Without proper administration of ascorbate, the plasma concentrations face severe limitations and will not result in proper concentrations of clinical interest. Direct intravenous administration offers instant plasma concentration at levels otherwise unachievable by oral administration. To observe the beneficial antiproliferative effects of ascorbate, plasma concentrations must achieve a high enough millimolar concentration. *In vivo* studies have shown that ascorbate levels from 5-mM to even 30-mM are achievable by intravenous administration (8, 10).

Meta-analyses have examined the effect of antioxidant oral supplementation on general health. They found a correlation between excessive oral supplementation of antioxidants, one of the supplements being ascorbate, to the development of cancer and gastrointestinal issues. A correlation was found between high levels of oral supplementation and the development of cancers such as adenocarcinoma and upper gastrointestinal cancers (1, 4).

The manner in which ascorbate is delivered, like any other drug, is key to the success of the molecule achieving therapeutic and curative effects. The history of ascorbate shows the importance of drug administration methods and the possible complications associated with improper treatment routes of administration. High oral doses of ascorbate may in fact lead to development of cancer and other gastrointestinal complications (1, 4). The key to ascorbates effect *in vivo* may lie in the proper administration intravenously, achieving the high levels of ascorbate, such as the 5-mM and 10-mM examined in this *in vitro* experimentation. Ascorbate is

likely to act purely as an adjuvant to cancer therapies, rather than the sole treatment, but still requires the aforementioned concentrations of clinical interest.

## Hypothesis

I hypothesized that proliferation of the breast epithelial cells would be decreased in a dose-dependent manner as millimolar concentrations of ascorbate increased. The greater presence of ascorbate in theory would overcome the cells' ability to withstand oxidative stress as well as afflict the glycolytic pathway, limiting energy availability. Glycolytic involvement is speculated and related to other studies; glycolysis activity was not measured in my experimentation. I also hypothesized that the introduction of the enzyme catalase would reverse the antiproliferative effects of ascorbate, resulting in an unchanged rate of proliferation in which I would see confluent growth in the flask that would resemble the 0-mM control flask. Negative results, the reversal of the antiproliferative effects of ascorbate, would support my hypothesis that ascorbate acts via a pro-oxidant mechanism to decrease the proliferation of the cell lines. Glycolytic enzyme activity and intermediates were not measured.

Due to technical difficulties within the lab, we were unable to complete research on gastrointestinal stromal tumor (GIST) cells, but the hypotheses would have been the same as those for the breast epithelial cells.

# Chapter 2

# Results

## MCF-10F Cell Line: Ascorbate

Figure 1. depicts the effect of ascorbate on the proliferation of the MCF-10F breast epithelial cell line. Baseline Day 0 was 0.040 O.D. 0-mM Day 1 was found to be 0.026 O.D. and exhibited a decrease in density to 0.153 O.D. by Day 2. 5-mM concentration of ascorbate exhibited a large drop to 0.00967 O.D. and rose slightly to 0.0103 O.D. by Day 2. 10-mM ascorbate concentration found a decrease to 0.0173 O.D. at Day 1. Day 2 found a decrease to 0.0137 OD. The MCF-10F cells exposed to 5-mM and 10-mM ascorbate had dramatically limited growth compared to the 0-mM control over the 3 day period of experimentation.

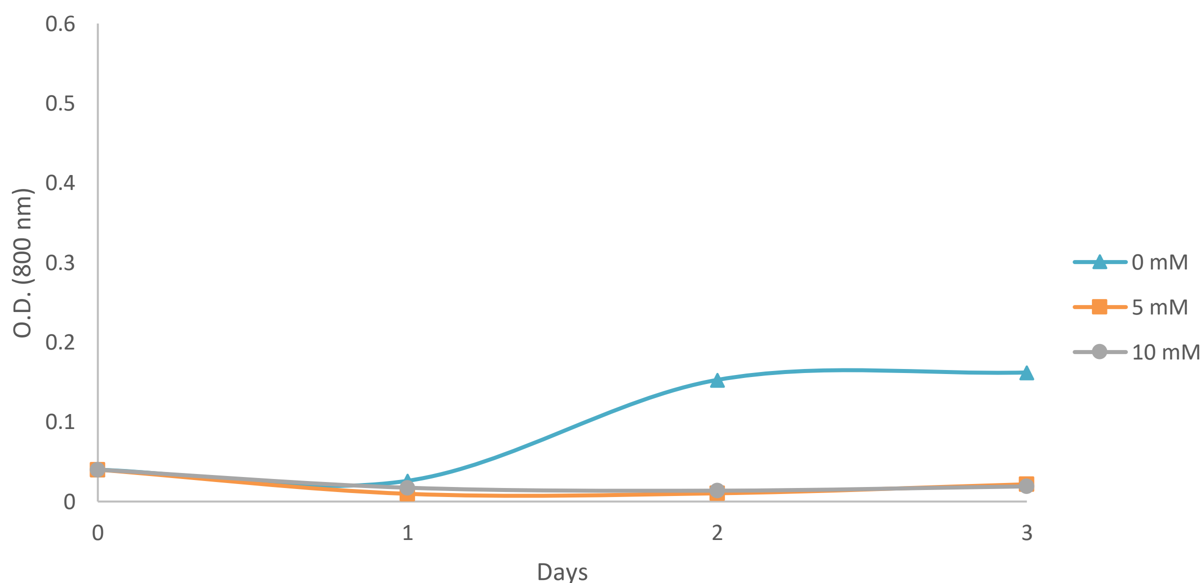


**Figure 1. Effect of varying concentrations of ascorbate on the proliferation of MCF-10F breast epithelial cell line.**

### MCF-10F Cell Line: Combination of Ascorbate and Catalase

Figure 2. examines the effect of catalase and ascorbate in combination on the proliferation of the MCF-10F breast epithelial cell line. Day 0 baseline was found to have an optical density of 0.198 O.D. 0-mM of ascorbate remained at 0.198 O.D. for Day 1 and began to rise to 0.363 O.D. by Day 2 and followed the upward trend to 0.541 O.D. by Day 3. 5-mM ascorbate initially dropped to 0.170 O.D. Day 1 and then grew to 0.310 O.D. by Day 3. 10-mM ascorbate had an initial large drop to 0.140 O.D. Day 1 and rose to 0.415 O.D. by Day 3. Overall, growth was more similar to the 0-mM control compared to solely ascorbate experiment. 10-mM proliferation was higher than that of the 5-mM group by Day 3.

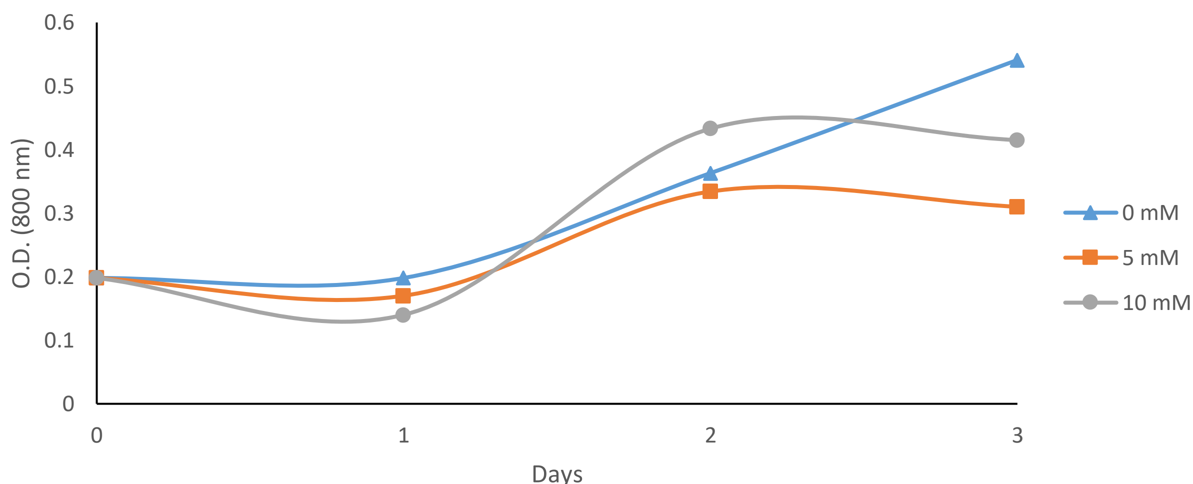


**Figure 2. Effect of catalase and ascorbate on the proliferation of MCF-10F breast epithelial cells.**

### E2 Cell Line: Ascorbate

Figure 3. shows the effect of varying concentrations of ascorbate on the proliferation of the E2 breast epithelial cell line. Day 0 baseline was found to be 0.391 O.D. 0-mM ascorbate

control rose to 0.414 O.D. Day 1. with a dramatic density increase to 1.022 O.D. by Day 3. 5-mM adsorbate at Day 2 saw a decrease to 0.293 O.D. and Day 3 saw an increase in optical density to 0.584 O.D. 10-mM ascorbate saw a 122% decrease to 0.176 O.D. by Day 1 and continued to drop to 0.162 O.D. by Day 3. We once again observed a limited cell growth compared to the control following exposure to 5-mM and 10-mM ascorbate concentrations exhibiting something similar to a dose-dependent effect.

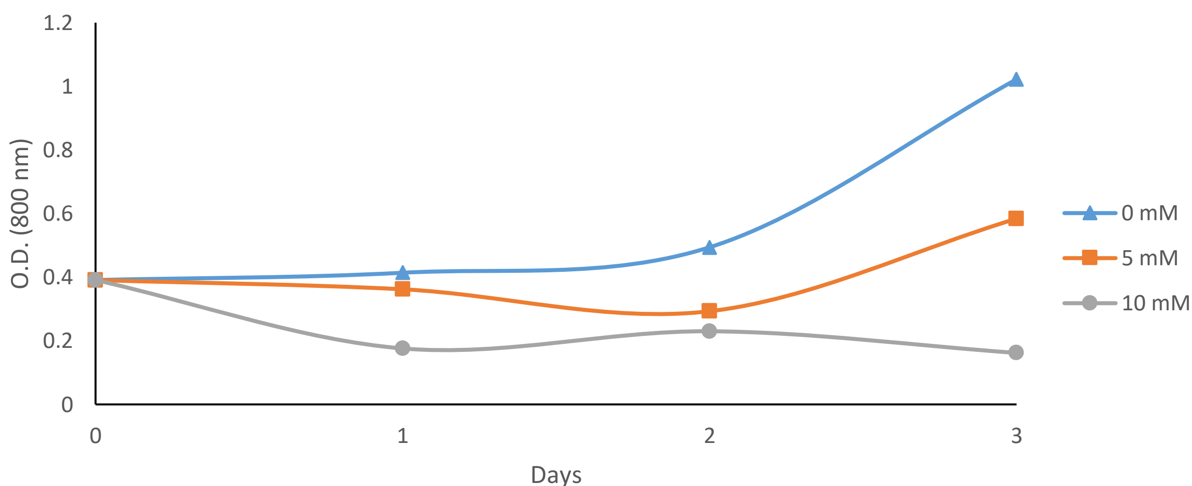


**Figure 3. Effect of varying concentrations of ascorbate on the proliferation of E2 breast epithelial cells.**

## E2 Cell Line: Combination of Ascorbate and Catalase

Figure 4. examines the effect of the effects on proliferation of the E2 breast epithelial cell lines with ascorbate in conjunction with catalase. Day 0 found a baseline of 0.154 O.D. 0-mM control group found an increase to 0.333 O.D. by Day 1 and subsequently rose to 0.786 O.D. by Day 3. The 5-mM ascorbate group found an increase to 0.350 O.D. by Day 1. Following a similar pattern to that of the control group, optical density rose to 0.775 O.D. by Day 3. The 10-

mM ascorbate group followed a similar pattern, rising to 0.277 O.D. by Day 1 and continued to rise to 0.766 O.D. by Day 3. We see a clear reversal of the antiproliferative effects of ascorbate regardless of ascorbate's concentration with the presence of catalase.

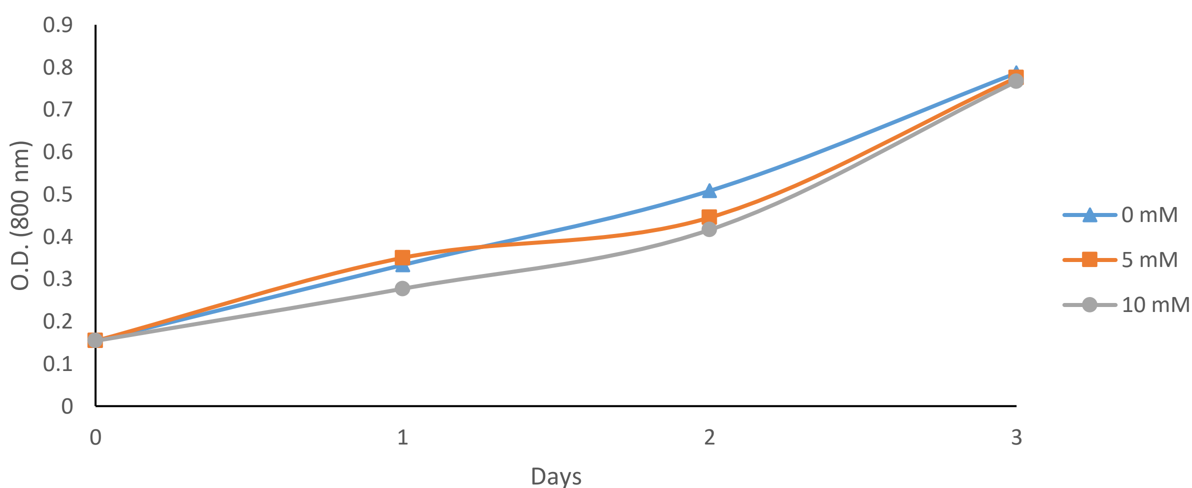


**Figure 4. Effect of catalase and ascorbate on the proliferation of E2 breast epithelial cells.**

## C5 Cell Line: Ascorbate

Figure 5. shows the effect of the varying concentrations of ascorbate on the proliferation of the C5 breast epithelial cell line. Day 0 baseline was found to be 0.160 O.D. Control 0-mM ascorbate group found an increase to 0.371 O.D. by Day 1 and continued to rise to 1.084 O.D. by Day 3. The 5-mM ascorbate group rose in a similar fashion to 0.358 O.D. by Day 1 and continued to show a low rise. Day 3 rose to 0.756 O.D. still well below the control group. The 10-mM ascorbate group rose slightly to 0.206 O.D. Day 1 and continued to rise very slowly resulting in a density of 0.331 O.D. by Day 3. The 10-mM ascorbate group resulted in the

greatest decrease in proliferation of the C5 cells. This appears to be strong evidence of a dose-dependent decrease in proliferation.

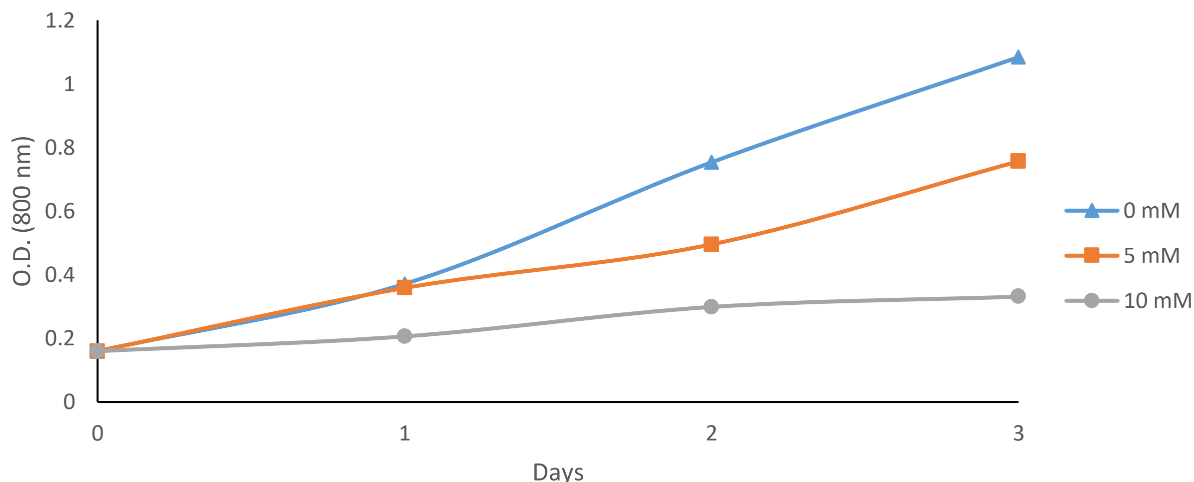


**Figure 5. Effect of varying concentrations of ascorbate on the proliferation of C5 breast epithelial cells.**

### C5 Cell Line: Combination of Ascorbate and Catalase

Figure 6. examines the effect of C5 cellular proliferation with exposure to both catalase and ascorbate. Day 0 baseline found an optical density of 0.261 O.D. The 0-mM group rose to 0.370 O.D. by Day 1 and continued to increase to 0.567 O.D. by Day 2. Day 3 found a final optical density of 0.894 O.D. The 5-mM ascorbate group rose to 0.444 O.D. by Day 1 and continued to rise to 0.786 O.D. by Day 3, close to the density of the 0-mM control group. 10-mM ascorbate group rose to 0.423 O.D. by Day 1. Day 2 found an increase to 0.751 O.D. and rose to 0.829 O.D. by Day 3. Oddly, the 5-mM and 10-mM groups appear to grow more than the control by Day 1 and 2, but catalase still appears to reverse the effects of ascorbate as observed with the final densities at Day 3.

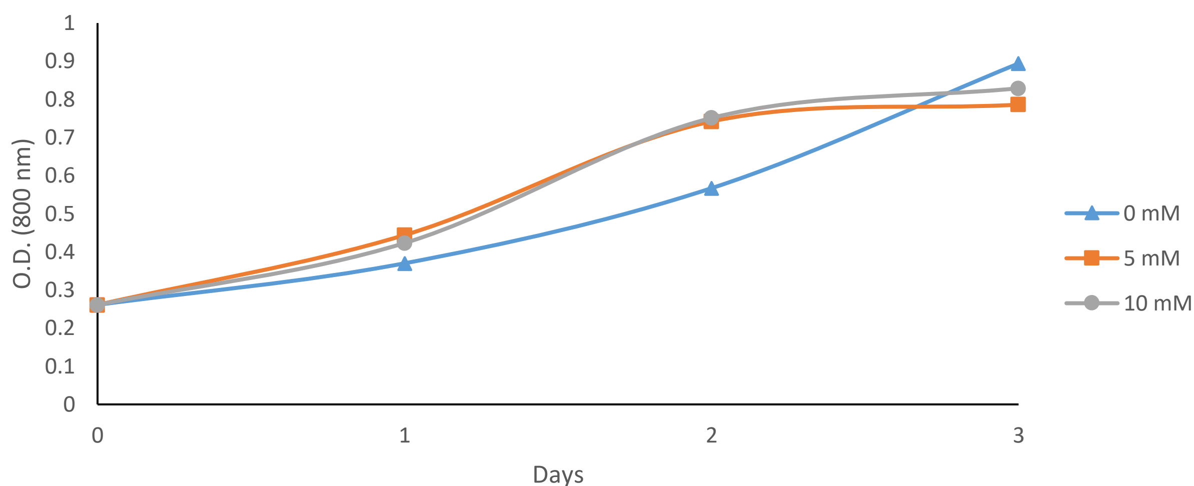


**Figure 6. Effect of catalase and ascorbate on the proliferation of C5 breast epithelial cells.**

## T4 Cell Line: Ascorbate

Figure 7. shows the effect of ascorbate on the proliferation of the T4 breast epithelial cell line. Day 0 found a baseline value of 0.165 O.D. The 0-mM control group rose to 0.240 O.D. by Day 1 and rose to 0.532 O.D. by Day 2. Day 3 showed a minor increase to 0.537 O.D. The 5-mM ascorbate showed an increase to 0.406 O.D. by Day 1. and by Day 3, there was a drop to 0.475 O.D. 10-mM ascorbate group rose to 0.339 O.D. Day 1 with a continued increase to 0.420 O.D. by Day 3. Again, we observed the oddity of the 5-mM and 10-mM group exhibiting a higher optical density by Day 1 than the control. The growth curve is different in comparison to previous cell lines, but close examination shows a similar dose-dependent decrease in proliferation by Day 3; statistical analysis would be required because it does appear as though growth by Day 3 is very similar in all concentration groups.

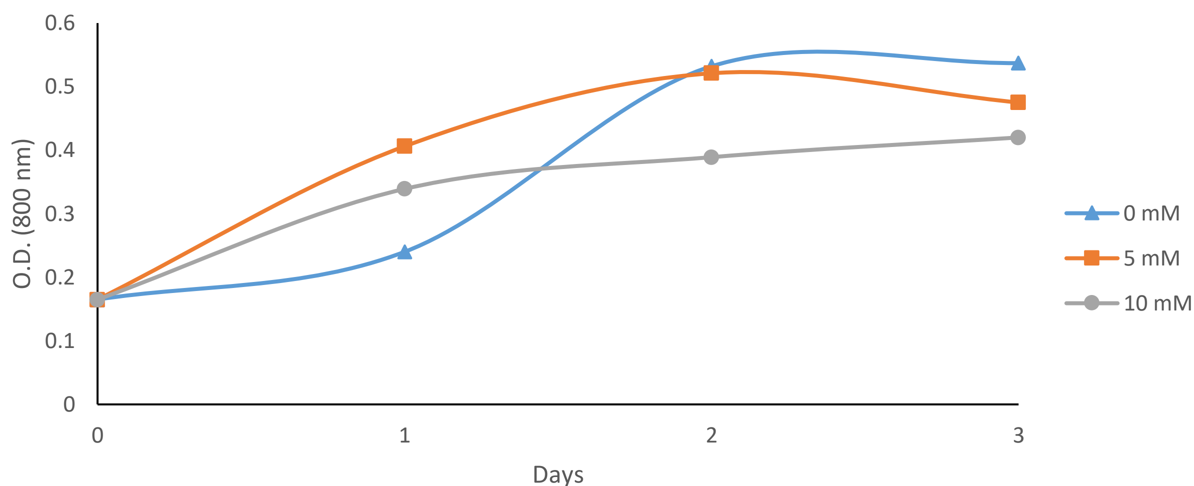


**Figure 7. Effect of varying concentrations of ascorbate on the proliferation of T4 breast epithelial cells.**

### T4 Cell Line: Combination of Ascorbate and Catalase

Figure 8. shows the effect of the presence of both ascorbate and catalase on the proliferation of the T4 breast epithelial cell line. Day 0 baseline was found to be 0.143 O.D. The 0-mM ascorbate group rose to 0.395 O.D. by Day 1 and rose to 0.499 O.D. by Day 2. Day 3 continued to rise to 0.591 O.D. 5-mM ascorbate group rose to 0.311 O.D. and continued to increase to 0.599 O.D. by Day 3. The 10-mM ascorbate group rose to 0.358 O.D. by Day 1 and found a final optical density of 0.544 O.D. by Day 3. This appears to show a much more controlled growth curve for all the groups in comparison to Figure 7 with the 5-mM group nearly identical to the control group in cellular density.

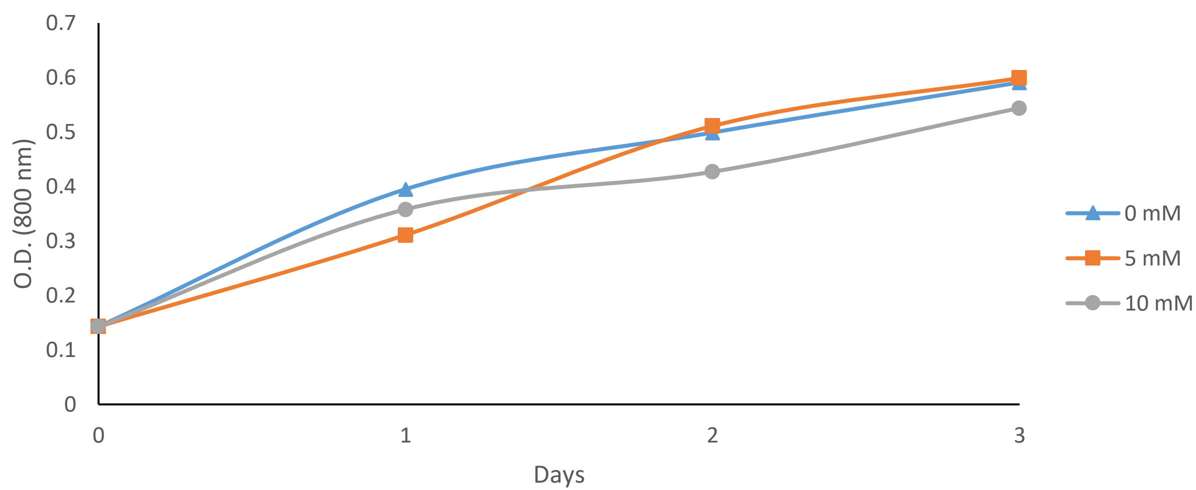


**Figure 8. Effect of catalase and ascorbate on the proliferation of T4 breast epithelial cells.**

**GIST Cell Line:**

Due to technical issues, GIST cells experimentation will no longer be reported on.

# Chapter 3

## Discussion

One of the most strongly supported mechanisms of action for ascorbate is via production of superoxides and free radicals such as hydrogen peroxide (9, 11). Free radicals will perturb the cancer cells redox homeostasis and make the oxidative stress unmanageable within the cells. Catalase is an enzyme of high interest due to its specificity toward hydrogen peroxide control. Cancer cells often lack or have a lower production of catalase than a normal, nonmalignant cell, making the malignant cell susceptible to oxidative stress (3).

The use of catalase in experimentation helped to isolate the likely free radical of action attributed to the observed changes in cellular proliferation; hydrogen peroxide concentration was not quantified. The use of spectrophotometry has been found to be an effective alternative method of cell counting without the time-consuming process of cell counting (7).

## Hydrogen Peroxide

Ascorbates most significant anti-cancer property is the production of hydrogen peroxide. Hydrogen peroxide is a potent oxidizer that will disrupt the redox balance within cells and even have a direct effect on cellular proteins and structures that may have a hand in promoting apoptosis (3, 5, 6). Hydrogen peroxide is likely produced through oxidation of ascorbic acid extracellularly, and possibly intracellularly once transported into the cell via GLUT1, via metal ions, such as iron, that are subsequently reduced that can then act to supply electrons to oxygen

species to create superoxides (13). These superoxides, if not scavenged, can be combined with hydrogen to form hydrogen peroxide (11). This production of hydrogen peroxide is often attributed as an extracellular mechanism, but may occur within the cell as well. Because of hydrogen peroxide's ability for oxidation, the supplementation of electrons to other species provides instability in whatever species accepts the electrons - electrons can often to be attributed to the reactivity and the energy state of different molecules. Redox reactions are key in the transfer of energy and can provide complications to different molecules if not controlled. The oxidation or reduction of a species can modify it to an intermediate of another molecule or increase chances of damage due to reactivity.

## Oxidative Stress

The production of free radicals, superoxides, as well as hydrogen peroxide leads to a severe amount of oxidative stress placed on the cell. Cancer's dependence on glycolysis as an energy source reduces the innate cellular oxidative stress. Cancer cells do not have a high dependence on the electron transport chain and oxidative phosphorylation for energy production like normal cells; cancer cells thus have a lower innate oxidative stress due to the limited activity of the electron transport chain. Of interest, is the limited expression and use of reactive oxygen species scavengers in cancer cells to stabilize the stress created by the redox reactions. Expression is likely lower due to decreased need for scavengers because of the limited use of oxidative phosphorylation in energy production.

A molecule of interest for cellular survival of oxidative stress is glutathione. Glutathione acts as an antioxidant, a reactive oxygen species scavenger to prevent excess damage within the

cell. Glutathione's ability to scavenge is overpowered due to ascorbate's ability to produce high concentrations of hydrogen peroxide that will result in cellular damage (10). This can be found in my results; as the concentration of ascorbate increases there is a clear dose-dependent decrease in cellular proliferation in all but the T4 cell line. This dose-dependent decrease in cellular proliferation is quite pronounced in the E2 and C5 lines with a distinct decrease in optical density. The catalase experimentation showed a relatively normalized cell growth, no longer exhibiting the pronounced dose-dependent decreased proliferation. This experimentation with catalase supports the concept of hydrogen peroxide acting to perturb the cells ability to handle the oxidative stress, but with introduction of catalase, the cell returns to a fairly normalized growth with a reversal of ascorbate effects.

Other studies have found no change in cellular proliferation in normal cells after exposure to ascorbate which is likely due to the effective expression and presence of required antioxidants and reactive oxygen species scavengers. Our results oddly observed a decrease in the MCF-10F proliferation though it is one of the more normal cell lines. Another study found no effectiveness of ascorbate in cellular proliferation, and in fact exhibited some general cytotoxicity to other nonmalignant cells, similar to what I found with the MCF-10F cell line (16). Another study has also found ascorbate to actually suppress the effect of chemotherapeutic drugs, contraindicative of the use of ascorbate (15).

## Glycolysis

Cancer cells often rely heavily on glycolysis as a prime means in which to produce energy, rather than a dependence on oxidative phosphorylation. Ascorbate has a structure very

similar to that of glucose and can be transported through the glucose transporters GLUT1 and GLUT3 (13, 17). This transport of ascorbate into the cytoplasm allows for access to the glycolytic cycle within the cell. Studies have found that induction of *KRAS* and *BRAF* mutations result in a greater expression of GLUT1 transporters, allowing for greater ferrying of ascorbate into the cell via dehydroascrobate, further supporting the possibility of an intracellular mechanism (17).

Ascorbate acts to suppress the activity of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). It has been found that the oxidation of GAPDH's residues inhibits its activity, in particular cysteine residues in the active site (14). GAPDH is a critical enzyme later in the glycolytic pathway (17). Inhibition of GAPDH activity via oxidation was found in the presence of hydrogen peroxide and metal ions, supporting the concept of ascorbate's production of hydrogen peroxide via reducing metal ions which, in turn, reduces oxygen to a superoxide that can then combine with hydrogen to form hydrogen peroxide (5).

Without GAPDH activity and the continuation of the glycolytic pathway, the cell loses the production of two ATP and will in fact invest two ATP to trap glucose within the cell and to create the proper fructose isomer. Without the production of ATP along with an energy investment, the cell is depleted of needed energy to proceed forward with growth. Because cancer cells are largely dependent on the activity of the glycolytic pathway rather than oxidative phosphorylation, the cancer cell is effectively depleted of energy. The energy crisis within the cell can lead to cellular death or a stunt in growth (3, 5, 6). A stunt in growth or cellular death could be attributed the observed lowered optical density following exposure to ascorbate.

## Negative Results of Catalase

The negative results we obtained through catalase experimentation support the idea of hydrogen peroxide oxidation. Many of the cells lines, largely excluding the T4 cell line, exhibited a normalized cell growth in comparison to exposure to ascorbate alone. This normalized growth is likely due to catalase neutralization of hydrogen peroxide which, in turn, creates a more stable redox environment; the stable redox environment allows for proper activity of GAPDH and the production of ATP. Hydrogen peroxides tendency for oxidation of other species may tie in the purpose of hydrogen peroxide from an enzymatic standpoint rather than just induction of oxidative stress on the entire cell.

# Chapter 4

## Conclusion

Based on my results, ascorbate has an anti-proliferative effect on breast epithelial cancer cells and acts via a pro-oxidant mechanism. We examined a decreased proliferation pattern in the optical density of three of four cell lines tested. The T4 cell line is the most aggressive of the lines and likely warrants a greater ascorbate concentration to have the same observable effects as seen with the other cell lines. Likely a 30-mM ascorbate exposure would be of interest because it is achievable *in vivo.*

The catalase experimentation and the observed negative results compared to the ascorbate-only experiments support the idea of a pro-oxidant mechanism. Catalase has a specificity toward hydrogen peroxide and its inclusion in the experiment resulted in a relatively normalized cell growth compared to the control 0-mM flask regardless of the concentration of ascorbate. Catalase effectively reversed the observed antiproliferative effects of solely ascorbate.

My hypothesis was supported for most of the cell lines. We observed a dose-dependent decrease in cellular proliferation in most of the cell lines. This is likely due to the presence of hydrogen peroxide that may alter the activity of glycolytic enzymes because of the results we obtained through catalase introduction. The introduction of catalase resulted in a relatively normalized cell growth relative to the 0-mM concentration group in the experiment, directly supporting the notion ascorbate acts via a pro-oxidant mechanism by hydrogen peroxide production as the free radical to disrupt cellular proliferation.

# Chapter 5

## Future Experiments

Due to technical issues within the lab throughout the experimentation period we were unable to observe the effects of ascorbate on GIST cells. It would be of interest to see if these cells as well as imatinib drug-resistant lines would be susceptible to ascorbate exposure. Also, due to the technical issues within the lab we were unable to run triplicate experiments on the breast cell lines. Without triplicate experimentation, we do not have the ability to run a statistical analysis.

It would be interesting to test ascorbate on cancer cell lines with known and defined cancer therapeutic drugs. Ascorbate is not a cure to cancer, but is likely an effective adjuvant for treatment. Other studies have found ascorbate to decrease the effectiveness of certain cancer therapy drugs which may result in a contraindication for the implementation for ascorbate treatment (15, 16).

It is odd that the MCF-10F breast epithelial cell line exhibited a large degree of decreased cellular proliferation because the MCF-10F are the most normal of the cell lines. The MCF-10F cells are immortalized and it would be of interest to find breast epithelial cell lines that are not. Previous studies found there to be little to no effect on cellular proliferation of nonmalignant cells with ascorbate concentrations, but my results showed a severe decrease in proliferation (8).

Of extreme interest, would be measuring glycolytic enzyme activity and the presence of intermediates and products. Hydrogen peroxide concentrations would be interesting to quantify and correlate it to the enzymatic function. I would expect that as hydrogen peroxide

concentration rises, due to the presence of higher concentrations of ascorbate, there would be lower activity of the glycolytic enzyme GAPDH.

# Chapter 6

# Materials and Methods

## Cells Lines

MCF-10F cells are an immortalized breast epithelial cell line. E2 cells were further derived from the MCF-10F lines via exposure to estradiol. C5 invasive cells were isolated from E2 cells that were put into a Boyden chamber and were then invaded to the fifth level of the membrane. This treatment allowed for the C5 cells lines to obtain invasive abilities and were quite aggressive and quick to grow. T4 cell line is the most invasive and aggressive tumorigenic line. All of these cell lines supplied by Dr. Jose Russo at Fox Chase Cancer Center.

## Ascorbate Experimentation Breast Epithelial Cells

Cells were cultured in high calcium medium in T-75 flasks, once the cells reached 80-90% confluency, the cells were split into T-25 flasks for experimentation. The breast epithelial cells were exposed to three different dosages of ascorbate: 0-mM (control), 5-mM, and 10-mM. The medium as well as ascorbate was refreshed daily throughout experimentation for a period of 3 days. Cells were extracted by trypsinization and centrifuged for 6 minutes at 4000 RPM. The cells were resuspended in 1 mL of culture medium and counted via optical density with the spectrophotometer at 800 nm as an effective alternative to direct cell counting (7).

## Ascorbate and Catalase Experimentation

The same procedure was followed as described above with the following addition. Catalase was added at a final concentration of 100 µg/mL and refreshed daily throughout experimentation for a period of 3 days.

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Major: Biology
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*Thesis Title:* Antiproliferative Effect of Ascorbate on Breast Epithelial Cells Acts via a Pro-Oxidant Mechanism of Hydrogen Peroxide Production

Grants Received: Abington College of Undergraduate Research Activities (*ACURA*) Grant

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## Work:

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Clinical Associate
Role as a nursing assistant, acting to help nurses with direct patient care on a Med/Surg floor within the hospital. Acquire vital signs, perform EKGs as well as other needed activities within my scope of practice.
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8/2015 - Present
Undergraduate Researcher
Under the supervision of Dr. Thomas McGuire worked in the laboratory at The Pennsylvania State University – Abington examining the effect of ascorbate on breast epithelial cells.
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