Ascorbate Decreases Proliferation of Breast Epithelial Cells and Acts Through a Pro-Oxidant Mechanism

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Abstract
Ascorbate has been shown to produce anti-proliferative effects on the growth of cancer cells. Three breast epithelial cell lines (MCF-10F, E2, and C5) were exposed to different dosages of ascorbate to determine its effect on the proliferation of these cells. Cell number was quantified using spectrophotometry. In a dose-dependent manner, ascorbate reduced proliferation of these breast epithelial cells in vitro. To determine whether ascorbate’s mechanism of action involved oxygen radical formation (including hydrogen peroxide), catalase was added in addition to ascorbate. Cell proliferation in the presence of catalase and ascorbate resulted in normalized cell growth suggesting that ascorbate acts as a pro-oxidant. Decreased proliferation of these cell lines suggests that ascorbate can be used therapeutically as an adjuvant to traditional chemotherapy.

Introduction
Cancer is a disease that involves abnormal cell growth which commonly results in death. Recently, it has been discovered that ascorbate (also known as vitamin C) slows the rate of growth of specific types of cancer cells, including prostate, pancreatic, hepatocellular, colon, mesothelioma, and neuroblastoma. Ascorbate may act as a pro-oxidant that creates free radicals that purportedly decrease the proliferation of the cancer cells. The free radical believed that is produced is hydrogen peroxide which can be broken down by catalase. Catalase is an enzyme that certain cancer cells lack or have limited production of. This may explain why the ascorbate is not cytotoxic to regular cells in other studies. Ascorbate has been used as an anti-cancer treatment in studies in the past, originally proposed by Linus Pauling. It has recently been shown to produce anti-proliferative effects on the growth of cancer cells when introduced to the body intravenously. However, when given orally, ascorbate does not produce the same effects because of the limitations of absorptions through the gastrointestinal tract.

Breast cancer is one of the leading causes of death in the United States of America. Since ascorbate has been shown to decrease proliferation of certain cancers, human breast epithelial cell lines (MCF-10F, E2, and C5) were tested to determine whether similar results would be observed.

Results

Figure 1. Effect of Ascorbate and Catalase on Proliferation of MCF-10F Breast Epithelial Cells. Left: Ascorbate only. Right: Ascorbate and catalase.

Figure 2. Effect of Ascorbate and Catalase on Proliferation of E2 Breast Epithelial Cells. Left: Ascorbate only. Right: Ascorbate and catalase.

Figure 3. Effect of Ascorbate and Catalase on Proliferation of C5 Breast Epithelial Cells. Left: Ascorbate only. Right: Ascorbate and catalase.

Discussion and Conclusions

- Ascorbate, in a dose dependent manner, decreases proliferation of MCF-10F and C5 breast epithelial cells in vitro.
- Decreased proliferation of MCF-10F and C5 breast epithelial cells suggests that ascorbate could be best used as an adjuvant to traditional chemotherapy as treatment.
- Reversal of anti-proliferative effect by catalase suggests that ascorbate acts, at least in part, as a pro-oxidant.

Future Experiments

- Use higher concentrations of ascorbate on the T4 cell line.
- Repeat all experiments in triplicate to replace pilot study results.
- Investigate whether anti-proliferative effects of ascorbate are also due to induction of apoptosis.

Materials and Methods

Cell Lines
- MCF-10F cells are an immortalized breast epithelial cell line.
- E2 cells were derived from MCF-10F cells by exposure to estradiol.
- C5 invasive cells were isolated from E2 cells that were put in a Boyden chamber and then invaded to the fifth level of membrane. Cells acquired invasive abilities from this treatment.

Ascorbate Acid Experiment
- Cells were grown in high calcium medium in T-25 and T-75 flasks.
- Exposed cells to three different dosages of ascorbate (0 mM, 5 mM, 10 mM) for a period of 0 to 3 days.
- Cells were extracted by trypsinization and centrifuged for 6 minutes at 4000 rpm.
- Cells were counted by spectrophotometry at 800 nm.