

# Ascorbic Acid and Copper are Effective Cytotoxic Agents Against Metastatic Breast Carcinoma Cells

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## Abstract

Ascorbic acid (Vitamin C) has been proposed as a non-toxic agent against cancer due to its redox capacity. In addition, copper is known to be a cofactor in many of the metabolic reactions mediated by ascorbic acid is also part of the protocol. Based on this information, we hypothesize that copper may enhance the killing effect of ascorbic acid in different metastatic breast carcinoma (BRCA) cells. To test this hypothesis, we exposed pleural-metastatic and bone metastatic BRCA cells to low (0.5-1 mg/ml), moderate (0.25-1.0 mg/ml), and high (2.5-5.0 mg/ml) doses of ascorbic acid from 24 to 72 hours. We also exposed the cells to a combination of ascorbic acid and copper (10 mcg/ml) to assess the effect of copper in cell growth. Quantity of cells (cell number) was determined by the MTS Assay. Results showed that: 1) High doses of ascorbic acid resulted in variable growth characteristics in both pleural and bone metastatic BRCA cell lines, suggesting complex metabolic intracellular mechanisms (i.e recycling of ascorbate-dehydroascorbate in a closed system). The addition of copper did not affect the variable result obtained from using ascorbic acid alone. 2) Moderate doses of ascorbic acid without copper had only a small, non-statistical effect on the growth of pleural and bone metastatic BRCA cells. Addition of copper caused a net cell killing effect ( $p > 0.05$ ). 3) Low doses of ascorbic acid with or without copper had not net effect on the growth of both metastatic BRCA cells lines. We conclude that: Ascorbic acid can exert a dose dependent effect on metastatic BRCA cells and copper enhances the killing effect at moderate doses of ascorbic acid. These results suggest that ascorbic acid, in combination with copper, have a potential as therapeutic agents in cancer treatment. These findings need further investigation at the molecular, biochemical, cellular and clinical levels.

## Introduction

Breast cancer is a common malignancy among women. It carries very high morbidity and mortality [1,2]. In addition current, systemic therapy produces toxicity. The cytotoxicity of chemotherapeutic agents limits their effectiveness in improving survival in cancer patients. Quality of life, as well as, the overall performance of the cancer patient is impaired with this therapeutic but highly toxic approach. Therefore, the possibility of the development of non-toxic therapies for cancer is very appealing. Nevertheless, the use of non-toxic cancer chemotherapy should be based on solid research and scientific data. There is a need for effective and less toxic systemic therapy. Ascorbic acid (Vitamin C, AA) has been proposed as an anticancer agent since the 50's [3]. It has recently been used in high intravenous concentrations in

clinical settings with improved survival rates among terminally ill cancer patients [4]. In general, ascorbic acid (AA) is an essential vitamin. Copper is a micronutrient identified as necessary for the coupling of biological reactions involving ascorbic acid. Ascorbic acid and copper, under the presence of oxygen, can produce hydrogen peroxide [5]. This oxidative species has been shown to be especially cytotoxic to solid tumor cells due to their lack of catalase [6]. This selective trait of solid tumor malignant cells can be successfully targeted for non-toxic chemotherapy.

## Methods

### Cell Culture

MDA-231 pleural-metastatic and MDA-231 F-10 bone metastatic

breast carcinoma cells were cultured in DMEM F-12, 5% Fetal Bovine Serum (FBS) and 1% glutamine. A total of 12,500 cell/well were incubated at 37 °C, 5% CO<sub>2</sub> for 4 hours. Afterwards, the media was decanted and replaced with 100 ml of the following solution:

- Control Group – DMEM F-12, 5% FBS glutamine.
- Copper Group – DMEM F-12, 5% FBS 1% glutamine, CuSO<sub>4</sub> 10 mg/ml.
- AA Alone Solutions Group – DMEM F-12, 5% FBS, 1% glutamine with AA in low concentrations (0.05 – 0.01 mg/ml), moderate (0.25 – 1.0 mg/ml) and high dose (2.5 – 5.0 mg/ml).
- AA Solutions Plus CuSO<sub>4</sub> Group – DMEM F-12, 5% FBS, 1% glutamine CuSO<sub>4</sub> 10mg/ml and AA in low dose (0.05 – 0.01 mg/ml), AA moderate dose (0.25 – 1.0 mg/ml) and high dose (2.5 – 5.0 mg/ml).

### Cell Number

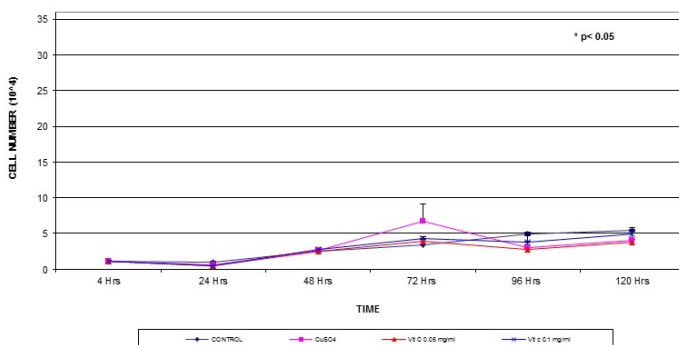
Determination of viable cell number was done utilizing Promega MTS Assay read at 490 nm wavelength, at time intervals from 4 to 120 hours. MTS reacts with metabolically active cells to produce a soluble chemical formazan, with when measured at 490 nm wavelength provides an absorbance that is proportional to cell number.

### Statistics

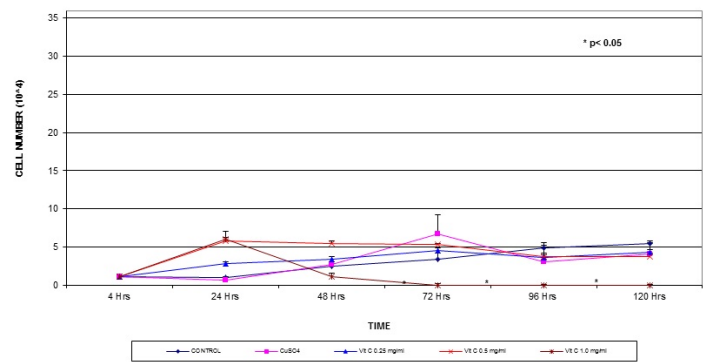
Two ways ANOVA was used to analyze the data and statistical significance was established at  $p < 0.05$ .

### Results

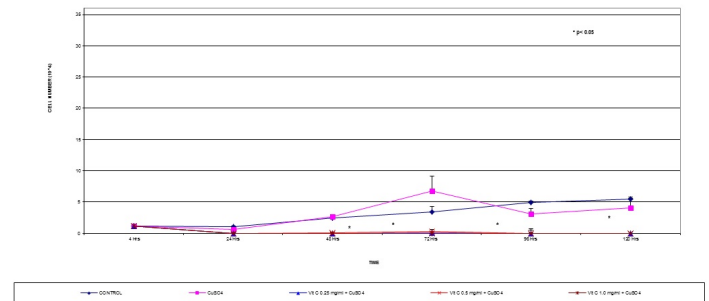
Low doses of AA (0.05 – 0.1 mg/ml) with or without copper had no net effect in the growth of metastatic breast cancer cells in this study (Figure 1). Moderate doses of AA (0.25 – 1.0 mg/ml) had no effect on the growth of metastatic breast cancer cells, except at the higher dose level (1.0 mg/ml) (Figure 2). Moderate doses of AA (0.25 – 1.0 mg/ml) in combination with CuSO<sub>4</sub> (10mg/ml) had a significant cell killing effect ( $p < 0.05$ ) (Figure 3). High doses of AA (2.5 – 5.0 mg/ml), either alone or with copper, had a variable effect upon cell growth (Figure 4). There was no difference in the growth parameters between the two metastatic breast cancer cell lines (bone or pleural) treated with AA alone or in combination with copper.



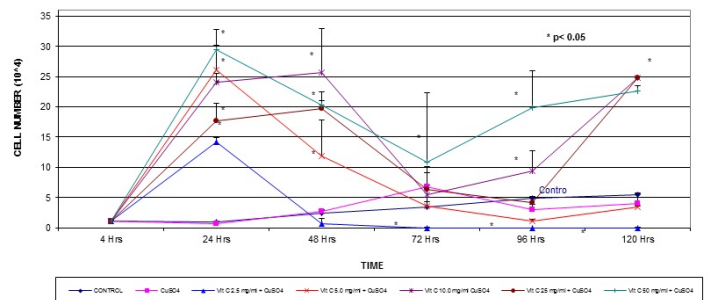
**Figure 1:** Effect of Ascorbic Acid (Vitamin C) in the Growth of Bone - Metastatic Breast Carcinoma Cells (Low Doses).



**Figure 2:** Effect of Ascorbic Acid (Vitamin C) in the Growth of Bone - Metastatic Breast Carcinoma Cells (Moderate Doses).



**Figure 3:** Effect of Ascorbic Acid (Vitamin C) and CuSO<sub>4</sub> in the Growth of Bone - Metastatic Breast Carcinoma Cells (Moderate Doses).



**Figure 4:** Effect of Ascorbic Acid (Vitamin C) and CuSO<sub>4</sub> in the Growth of Bone -Metastatic Breast Carcinoma Cells (High Doses).

### Discussion

As we expected, low dose of AA had no effect on malignant cell growth either with or without copper in our in vitro model. Low dose of AA (0.05-0.1 mg/ml) probably was not high enough to produce significant oxidative species (mainly hydrogen peroxide, H<sub>2</sub>O<sub>2</sub>) to decrease malignant cell proliferation. In fact, there is literature pointing out that ascorbate in low levels is stimulatory to cell proliferation, as well as data on copper and hydrogen peroxide [7-10].

The moderate doses of AA (0.25 – 1.0 mg/ml) had an inhibitory effect on malignant cell proliferation which can be seen in morphological evaluations. This dose of AA seems to reach the necessary level to attain an inhibitory growth activity. The moderate

doses of AA with added copper had a significant cell killing effect in our in vitro model. Ascorbate can generate hydrogen peroxide upon oxidation with oxygen in biological systems (especially in the presence of copper) [11,12]. Hydrogen peroxide can generate enough damage to cause cell death [6]. Also, hydrogen peroxide may further generate additional reactive species such as the hydroxyl radical and aldehydes which can compromise cell viability [13].

High doses of AA (2.5-5.0 mg/ml) either alone or with copper had a variable effect on cell proliferation and death. This AA concentration had a biphasic effect on tumor cell proliferation probably due to back and forth redox reactions between AA and dehydroascorbic acid (DHA) in a closed system. This resonance activity observed in our study is probably nonexistent in open systems where the probability of AA and DHA molecule collision is highly reduced.

### Conclusion

AA exerts a dose dependent effect on the growth of pleural and bone metastatic breast carcinoma cells. Copper (as CuSO<sub>4</sub> 10mg/ml) enhances AA cell Killing effect in both metastatic cell lines. AA is a potential chemotherapeutic agent with relatively no systemic toxicity. These results need further evaluation.

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