CANCER RESEARCH

Inhibition of Human Breast Carcinoma Cell Proliferation by Ascorbate and Copper

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We tested the effect of different concentrations of ascorbic acid (AA), 50, 100, 250 mg/500 mg/dL) with copper sulfate (CS), 10 mg/dL) on human breast carcinoma (MDA-MB231) cell proliferation *in vitro*. Cell proliferation was measured using a colori- metric assay (Cell proliferation kit II (XTT), Boehringer, NJ). The results of the mean absorbance of the tissue culture at different AA concentrations and a constant CS concentration were as follow: 0.82±0.03 (control, mean±SE), 0.64 ± 0.02 (CS above); 0.48 ± 0.03 (50 mg/dL) AA), 0.21± 0.02 (100 mg/dL), 0.08 ± 0.01 (250 mg/dL) AA, 0.60 ± 0.05 (500 mg/dL). These results show that a combination of AA and CS inhibits human breast carcinoma cell proliferation in vitro. This cell proliferation inhibitory effect is directly proportional to the AA concentration with the exception of the 500 mg/dL AA dose. This chemotherapeutic effect was optimally enhanced when AA was added at a concentration of 250 mg/dL. The AA concentrations of 500 mg/dL had a biphasic effect on tumor cell proliferation probably due to back and forth redox reactions between AA and dehydroascorbic acid in a closed system. This study provides preliminary evidence that AA and SC can be used as biological response modifiers (BRM) for tumor growth inhibition.

Key words: Vitamin C, Koper, Cancer

Effective treatment of solid tumors and there metastases has been extremely toxic and with limited success. Moreover, in the last two decades different combination protocols have not changed disease-free survival and total survival (1, 2). Current cancer therapy focuses on cell killing by directly attacking the cell's reproductive cycle, combining several highly toxic agents that have an array of secondary adverse effects. Little attention has been given to manipulating the local environment where these malignant cells develop as a means of treatment. This environment favors uncontrolled cell reproduction, tumor growth and metastasis probably due to a low generation of oxidative species (3). Cellular environmental conditions highly depend on intermediary metabolism, which can be influenced by biological response modifiers (BRM). It is known that solid tumor cells have a reduced concentration of catalase; this particular deficiency increases their susceptibility to oxidative reactions (4, 5). Thus, nontoxic chemotherapy consisting of the addition of pro-oxidant cations, vitamins with oxidation-reduction potential and certain fatty acids that may generate cystostatic and/or cytotoxic effects would be expected to produce a significant suppression of tumor growth. In order to explore this possibility, we embarked on preliminary studies utilizing ascorbic acid (AA) and copper as potential BRM for cancer due to their redox potential.

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Methods

Human breast carcinoma cells MDA-MB231 were prepared for cell culture using D-MEM/F12 media with antibiotic/antimycotic solution and fetal bovine serum. The cell proliferation study in human breast carcinoma cell lines MDA-MB231, Metastatic to pleura and metastatic to bone, exposed to (CS), 10 mg/dL and different concentrations of AA (50, 100, 250 and 500 mg/dL) was done under normal (ambient, 21%) oxygen conditions. Cell

proliferation was quantified by using the Cell Proliferation Kit II (XTT) colorimetric assay, (Roche-Boehringer Mannheim, NJ). Forty cell culture plates were prepared in triplicates and for each absorbance were measured as a mean to quantify cell proliferation for a period of 15 days. Mean, standard deviation, standard error and coefficient of variation were calculated and subjected to ANOVA statistical analysis.

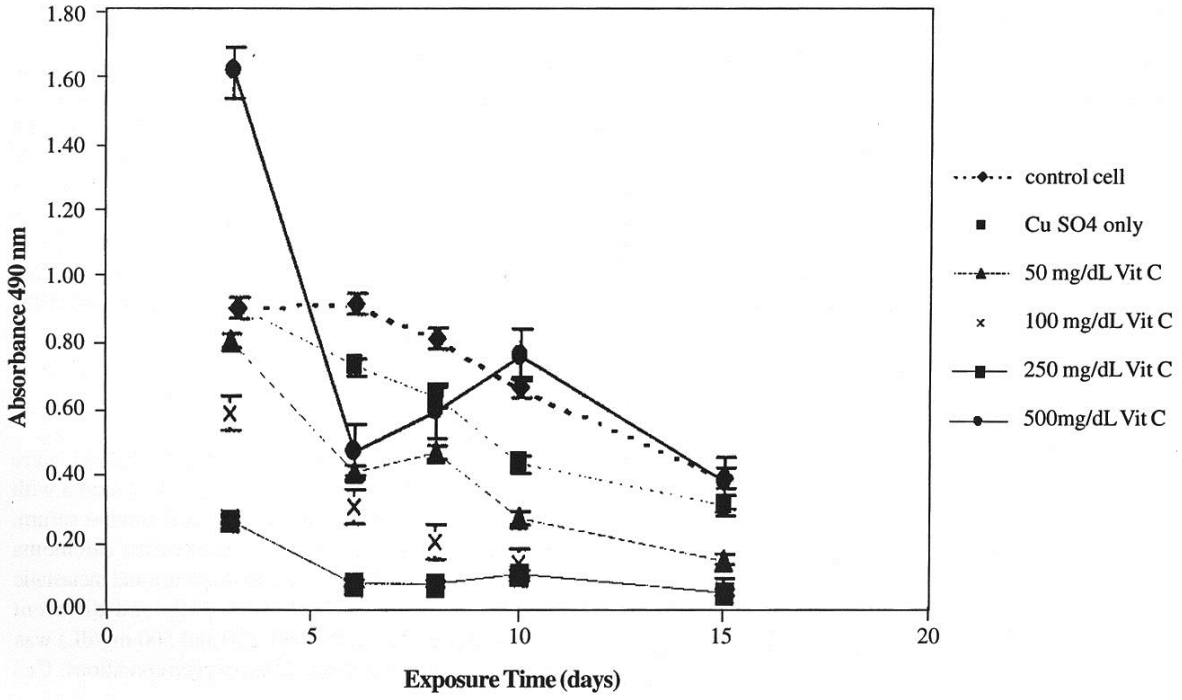
Results

The results of the proliferation study of the human breast carcinoma cell lines cultured at different AA and a constant CS concentration were as follow: 0.82±0.003 control mean ±SE), 0.64 ± 0.02 (CS above 0.48 ± 0.03 (50 mg/dL AA), 0.21 ± 0.02 (100 mg/dL) AA), 0.08 ± 0.01 (250 mg/dL AA), 0.60 ± 0.05 (500 mg/dL AA). The differences were statistically significant ($P < 0.05$). This cell proliferation inhibitory effect is directly proportional to the ascorbate concentration with the exception of the 500 mg/dL AA dose. Thus, a synergistic chemotherapeutic effect of AA and CS was optimally enhanced when AA was added at a concentration of 250 mg/dL. The AA concentration of 500 mg/dL had a biphasic effect in relation to of cell proliferation.

Discussion

AA is well known for its antioxidant properties, nevertheless due to its redox potential it may have an oxidation effect. This action is further enhanced by divalent cations such as copper (6). In the presence of free transition metal catalysts, AA oxidation can yield highly reactive species capable of initiating oxidative damage to the cells. Although not commonly occurring *in vivo*, its occurrence is possible in certain pathological states, such as cancer. We believe this pro-oxidant activity exhibited by AA is the main mechanism by which AA inhibits malignant cell proliferation (7). A quantitative difference (10-100 fold) has been reported in the content of the enzyme catalase between normal and malignant cells (4, 5). This relative deficiency of catalase present in the malignant cells makes them highly susceptible to oxidative damage induced by hydrogen peroxide. AA stimulated by copper releases two hydrogen atoms that react with oxygen and form hydrogen peroxide in the cellular aqueous environment. The MDA-MB231 (both pleural and bone metastatic types) used as control grew normally. The addition of either AA or CS alone slightly, but not significantly inhibited the growth of both malignant cell lines. AA at a dose 50 mg/dL plus CS enhanced the

Figure 1. Cell Line = MDA 231 F10 Bone
Cell density = 1.0 x 10^6



inhibitory effect. Addition of AA at a dose of 100 mg/dL resulted in a more pronounced inhibitory action than the 50mg/dL AA dose. The 250 mg/dL AA dose proved to be the most effective inhibitory dose in our *in vitro* system. The curve describing the absorbance of the 500 mg/dL AA dose shows an initial decline in absorbance (decrease proliferation) during days 3,4 and 6 followed by an elevation (increase in proliferation) during days 7 and 8 (Fig.1). This biphasic effect on tumor cell proliferation can be explained by AA's dual action as antioxidant and pro-oxidant. This initial inhibitory cell proliferation effect exhibited by the 500 mg/dL AA dose was probably due to its oxidation to dehydroascorbate and consequent production of hydrogen peroxide. In contrast, the enhanced cell proliferative activity exhibited afterwards was probably due to AA reduction and re-conversion back to dehydroascorbate in which a decrease in oxidative environment occurs and no hydrogen peroxide production is attained.

This effect was not observed at lower concentrations of AA because saturation level of AA in the cell environment is not achieved at these smaller concentrations. We should also mention that to certain extent, our system is a closed system which retains AA in the environment which permits this redox re-conversion to dehydroascorbate. It is conceivable that if cells, given a 500 mg/dL AA dose, are exposed to an hyper-oxygen environment, results would be similar to the 250 mg/dL AA dose ,since re-conversion to AA would be unlikely.

This study provides preliminary evidence that AA and CS may be used as BRM for tumor growth inhibition. This result brings into context a possibility of developing a non- toxic chemotherapy for cancer. Future studies are necessary to further explore this hopeful possibility.

Resumen

El efecto de diferentes concentraciones de ácido ascórbico (AA 50; 100, 250, 500mg/dl) se examinaron en la proliferación de células de carcinoma del seno humano (MDA-MB-231) *in vitro*. La proliferación celular se determinó utilizando un ensayo colorimétrico. Los resultados del promedio de absorbancia fueron los siguientes: 0.82 $\pm$ 0.03, control (promedio y error estandarizado), 0.64 $\pm$ 0.02 (SC),0.48 $\pm$ 0.03 (50 mg/dl AA), 0.21 $\pm$ 0.02 (100mg/dl AA), 0.08 $\pm$ 0.01 (250mg/dl AA) y 0.60 $\pm$ 0.05 (500mg/dl AA). Estos resultados demuestran que la combinación de AA y SC inhibe la proliferación celular del carcinoma de seno humano *in vitro*. El efecto inhibidor de proliferación celular fue directamente proporcional a la concentración de AA con la excepción de la dosis de 500mg/dl AA. Este efecto quimioterapéutico fue óptimamente aumentado en la dosis de 250mg/dl AA. La concentración de AA de 500mg/dl tuvo un efecto bifásico en la proliferación de células tumorales posiblemente debido al equilibrio dinámico de reacciones de oxido-reducción entre AA y el ácido dehidroascórbico en un sistema cerrado (*in vitro*). Este estudio provee evidencia preliminar que el AA y SC pueden ser usados como modificadores de la respuesta biológica (MRB) inhibiendo el crecimiento tumoral.

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