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Effect of ascorbic acid treatment with angiogenesis

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Abstract

Vitamin C plays is a cofactor for enzymes involved in many processes and has effects that are important for cancer transformation, such as antioxidant defense, transcription, and epigenetic regulation of gene expression. Angiogenesis is a normal process required for normal tissue repair and growth. Pathological angiogenesis is characterized by the persistent proliferation of endothelial cells and formation of blood vessels. The current study evaluated the effect of ascorbic acid on angiogenesis by investigating the expression of genes related to angiogenesis after treatment with different doses of ascorbic acid. By changing the concentration and administration time of ascorbic acid, a positive effect on the growth and metastasis of cancer cells in the group injected with ascorbic acid prior to having cancer cells injected into the abdominal cavity.

INTRODUCTION

New approaches and new carcinostatic agents have been developed, but their effects on cancer patients are not sufficiently known. One such agent is ascorbic acid. Vitamin C plays a role in the development and regulation of cancer growth and has been a topic of investigation and discussion for decades (1). It is a cofactor for enzymes involved in various processes and has important effects on cancer transformation, such as antioxidant defense, transcription, and the epigenetic regulation of gene expression (2). Vitamin C is also reported to exert beneficial effects on the immune system and inflammation, which is crucial in fighting precancerous and cancer cells by the host (3). The anticancer potential of vitamin C has been suggested by the results of many laboratory studies on experimental animals and cell cultures. The cytotoxicity of ascorbic acid on cancer cells reflects the oxidative stress resulting from the H2O2 generated in cell culture medium when ascorbate is present at concentrations of 1 mM or above, manifests as increased cell cycle arrest, p53 upregulation, decreased ATP levels, compromised mitochondrial function, suppression of antioxidant gene expression NrF-2, and/or cell death by apoptosis (4). The mechanism of action at these low concentrations remains unclear but potentially involves modification of cell survival pathways involving p53 (5). It has been shown that ascorbic acid has a definite effect as an antitumor agent when administrated at high dose concentrations. Provided intravenously, high-doses ascorbic acid works as a pro-oxidant therapeutic agent against cancer by generating ascorbate radicals and hydrogen peroxide in extracellular fluid in vivo. In addition, clinical case reports (from kidney cancer and bladder tumors) strongly indicate that high-dose ascorbic acid therapy in cancer treatment should be reassessed (6). These studies were examined and confirmed by histopathologic review. It has also been specified that high-dose ascorbic acid inhibits the cell migration ability and gap-filling capacity of endothelial progenitor cells (EPCs). Angiogenesis is a normal process required for normal tissue repair and growth. Pathological angiogenesis is characterized by the persistent proliferation of endothelial cells and formation of blood vessels (7). This complex process plays an important role in tumor growth, invasion, and metastasis. There have been conflicting results from studies evaluating the effect of ascorbic acid on angiogenesis during tumor development (8). The effect of low-dose ascorbic acid obtained from dietary concentrations was analyzed for tumor development in an animal model. The absolute number of blood vessels was reduced in animals with ascorbic acid-depleted tumors compared to fully supplemented animals (9). In contrast, another group found tumor angiogenesis to be independent of collagen synthesis and scorbutic levels of ascorbic acid. Conversely, high concentrations of ascorbic acid administered to cauterized corneas was found to suppress angiogenesis in a rat model. The current study evaluated the effect of ascorbic acid on

angiogenesis by investigating the expression of genes related to angiogenesis after treatment with different doses of ascorbic acid.

METHODS AND MATERIALS

The MCF7 cell lines provided by the Pasteur Institute of Iran were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco, USA), 100 U/ml penicillin-streptomycin (Sigma) and non-essential amino acids (Sigma), at 37 °C in a 5% CO2 atmosphere. Female BALB/c mice weighing 1513 g were kept under standard laboratory conditions (tap water, constant room temperature 22 °C). 5 × 10^5 cells in 200 ml PBS were injected into the breasts of the experimental mice using a 21 G injector. BALB/C mice were divided into 6 groups with 3 mice per group. Group A was the control group that was treated with phosphate buffer saline (PBS). Group B was treated with low-level ascorbic acid; Group C was treated with high-dose ascorbic acid; Group D was MCF7 cells for cancer induction; Group E received both cancer cells and high-dose ascorbic acid; and Group F received both cancer cells and low-dose ascorbic acid. The high dose of ascorbic acid was 1.7 × 10^-4 mol (30 mg), corresponding to 100 g for a human of 70 kg. The low dose of ascorbic acid was 3.1 ×10^-5 mol (5.5 mg). Twenty-four days after the initial treatment, the breast tissue of the mice was removed and kept at -70 °C until RNA was extracted using the FavorPrep™ Tissue Total RNA mini-kit (Favorgen, Taiwan). DNA was synthesized from 5 µg of total RNA using the BioFact™ RT kit (Biofact, South Korea). PCR amplification was done in a 20-µl total volume by BioFact™ 2X Real-Time PCR Master Mix (Biofact, South Korea) and specific primer in Table 1 for bFGF, VEGF, and GAPDH as reference genes. PCR cycling parameters were 40 cycles for 5 min at 94 °C, 10 s at 94 °C, 15 s at 60 °C, and 20 s at 72 °C.

Results

Angiogenesis is an important mechanism in the genesis and growth process of cancer. This study analyzed the expression of genes involved in angiogenesis using quantitative real-time RT PCR. In Group D, the expression of bFGF was increased by about 16 times over that of the groups that did not receive injected cancer cells. This increase was 2.1 times and 1.6 times greater than the increase seen in Groups E and F, respectively (groups which had been treated with ascorbic acid after cancer cells were injected). In Group D, the expression of VEGF was increased 5.5 times. The expression of angiogenesis-related genes was remarkably reduced in the groups treated with ascorbic acid compared to the group with cancer cell treatment only. These results suggest that ascorbic acid in high concentrations inhibits angiogenesis by inhibiting the expression of angiogenesis-related genes.

Discussion

Ascorbic acid (vitamin C) is an essential dietary requirement in humans. Deficiency in ascorbic acid develops into scurvy, a disease that came into prominence in the era of long sea voyages and was first systematically described by Lind in 1753 (10). The anti-cancer mechanism of high-dose ascorbic acid has been reviewed in numerous papers. The mechanism by which high-dose ascorbic acid induces cytotoxicity in tumor cells remains controversial. The most common theory relates to its oxidation-reduction properties. In the presence of oxygen, ascorbic acid undergoes spontaneous oxidation, giving rise to dehydroascorbic acid and the superoxide (11). Clinical manifestations of scurvy reflect the crucial importance of ascorbic acid in angiogenesis and blood vessel repair. Angiogenesis is the physiological process by which new blood vessels are generated. Breast tumors and many other solid tumors require this fundamental step in order to grow beyond a few millimeters in diameter (12). Vascular endothelial growth factor (VEGF) is a potent and selective endothelial mitogen able to induce a rapid and complete angiogenic response in normal and malignant tissues by generating new blood vessels. In addition to being secreted by a number of different cell types, VEGF is over-expressed not only by breast cancer cells, but also by activated breast stromal cells, suggesting an active role for the latter in tumor growth and angiogenesis (13). Conflicting results have been reported by studies evaluating the effect of ascorbic acid on angiogenesis during tumor development. The effect of low concentrations of ascorbic acid obtained from dietary concentrations was analyzed for tumor development in an animal model. The absolute number of blood vessels was reduced in ascorbic acid-depleted tumors compared to fully supplemented animals. In contrast, another study found tumor angiogenesis to be independent of collagen synthesis and scorbutic levels of ascorbic acid (14). In the current study, no difference in tumor growth was detected between the ascorbic acid-depleted tumor and the fully supplemented ascorbic acid mouse groups. Conversely, high-dose ascorbic acid administered to cauterized corneas was found to suppress angiogenesis in a rat model. Angiogenesis-related genes are directly involved in the growth and metastasis of tumors (15). It has previously been shown that expression changes in the angiogenesis-related genes bFGF and VEGF are closely related to tumor growth and metastasis. Therefore, this study investigated whether ascorbic acid reduced the expression of these two genes when used preventively and/or therapeutically (16). The results indicated that the expression of angiogenesis-related genes was lower in the cancer group than

in the group which received ascorbic acid after the induction of cancer cells. Roomi et al. (2006) reported similar results from in vitro and in vivo experiments (17). They observed changes in angiogenesis-related gene expression as an anticancer effect of ascorbic acid, lysine, proline, arginine, and green tea extract on various cancer cells and suggested that such substances, including ascorbic acid, were affordable as cancer remedies (18). By changing the concentration of ascorbic acid and the time it was administered, their experiments revealed the positive effect of ascorbic acid on the growth and metastasis of cancer cells in the group to which it was injected before cancer cells were injected into the abdominal cavity. Based on these experimental results, more clinical experiments as well as additional research on other cancers should be conducted.

Table 1. RT-qPCR Primer sequence for bFGF and VEGF

Primer name	Primer sequence
bFGF	5'-CGG CTG GCT TCT AAG TG-3'
	5'-CCC GTT TTG GAT CCG AGT TT-3'
VEGF	5'-ACA CGG GAG ACA ATG GGA TG-3'
	5'-TCT TGA CTC AGG GCC AGG AA-3'
GAPDH	5'-TTG CAG TGG CAA AGT GGAGA-3'
	5'-GGC TTC CCG TTG ATG ACA AG-3'

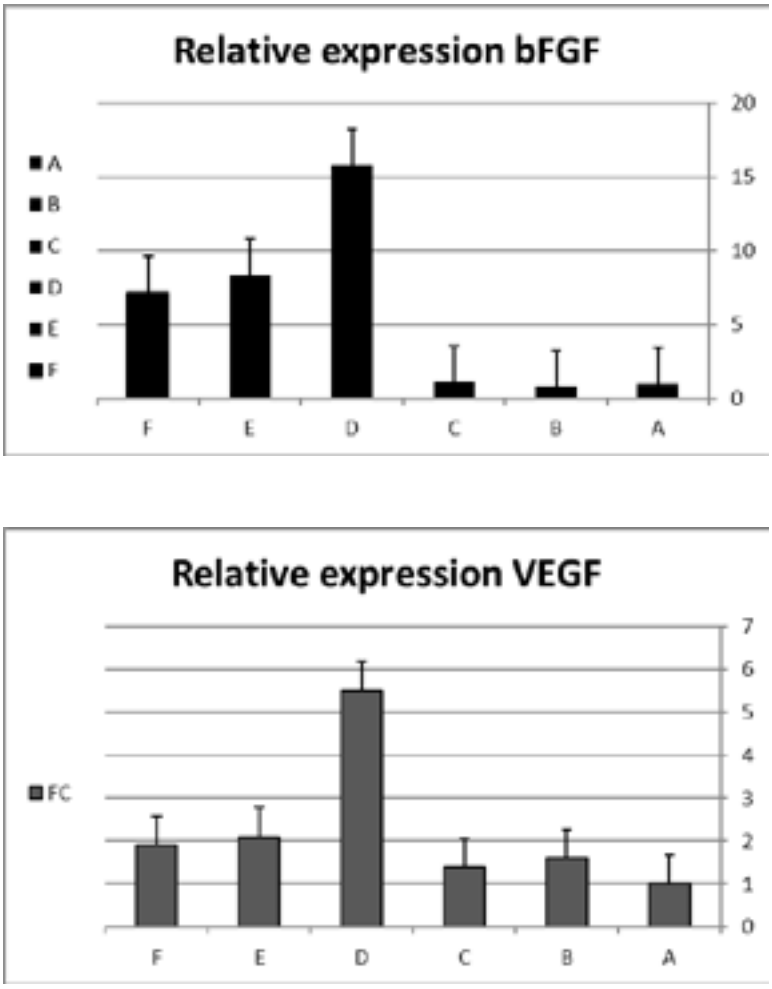

Fig. 1. Quantitative real time RT-PCR (qRT-PCR) analysis of the two angiogenesis related genes. Expression patterns of two angiogenesis related genes (bFGF and VEGF) were high in group D. Ascorbic acid treated groups showed suppressed expression of these genes. Each qRT-PCR is a representative example of data from 3 replicate experiments

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