

The effect of antioxidants in Ehrlich Ascites Cancer

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Abstract: A fundamental goal in molecular oncology is to unravel the underlying mechanisms which cause the cell transformation. In line with this approach, genome-wide functional screening approaches have revealed exciting insights into heterogeneous nature of cancer. Rapidly expanding horizons of research have unraveled myriad of pathways which play instrumental role in carcinogenesis and metastasis. Oxidative stress has also been reported to be significantly involved in cancer onset and progression. In line with this approach, oxidative stress modulating chemicals have always been sharply divided into antioxidants and oxidative stress-inducing agents. Conceptual and experimental advancements have enabled us to critically analyze full potential of these two different groups of chemicals in cancer chemoprevention. Different antioxidants are currently being analyzed in different phases of clinical trials. Although it has been reported in the literature that antioxidant supplements reduce tumor cells in some tumors or cause volume reduction in solid tumor sizes, there is no definite consensus. Therefore, an antioxidant supplement guideline based on more detailed clinical research and as a result of these is needed to achieve the best care for cancer patients and to avoid risky treatments for cancer patients.

Key words: Cancer; Antioxidants; Plants; Tumor.

Introduction

Experimental tumor modeling is of great importance in cancer research. Ehrlich Ascites Carcinoma (EAC), one of the experimental tumor models, is defined as a spontaneous breast adenocarcinoma. The EAC model is widely used in the literature on tumor pathogenesis and for the development of tumor suppressor agents (1, 2). EAC is often preferred in cancer research because of its capability to grow in the peritoneal cavity, form solid tumors in the skin and muscles, allow the inoculation of a standard number of cells, and measure the growth and shrinkage of the tumor mass (3, 4). EAC is initially hyperdiploid with being an undifferentiated carcinoma. As the virulence of this tumor increases through repeated passages, the rate of proliferation of such carcinomas increases gradually. Cells that become hetero-transplantable turn into the acid form. EAC is similar to the human tumors most susceptible to chemotherapy in that it is undifferentiated and has rapid growth rates (5, 6).

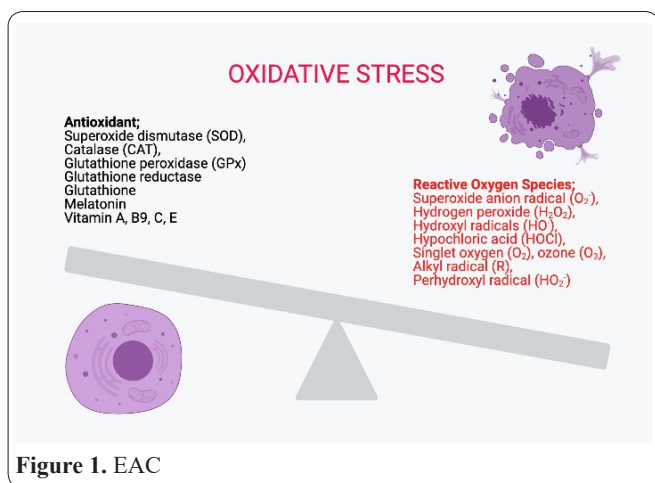
Evaluation of antioxidants in different phases of clinical trials provides significant clues about cancer chemopreventive roles. Natural compounds such as vitamins C, vitamin E, selenium, lycopene, carotenoids, soy products and green tea extracts are examples of substances having significant antioxidant and cancer chemo-

preventive properties (7). The use of antioxidants primarily targets the oxidative stress mechanism that plays a role in the pathogenesis of diseases such as cancer. Although oxidative stress occurs due to ionizing radiation, UV rays, smoking, exposure of biological systems to xenobiotics and the development of some pathological conditions, it is an important pathology that increases the production of free radicals (8). It is known that cellular damage caused by free radicals leads to the formation of cancer, cardiovascular diseases and neurodegenerative diseases. In the context of the importance of oxidative stress in diseases, free radical formation is tried to be controlled by various natural antioxidants.

Natural antioxidants have the ability to stabilize, neutralize or scavenge free radicals before they cause cellular damage (9). In this context, it is important to clearly determine the mechanism of action of natural antioxidants examined in cancer studies.

The effect of antioxidant property of flavonoids and other plant extract EAC

The role of various natural antioxidants in carcinoma development is summarized in EAC, which represents a chemotherapy resistant tumor model. Mechanisms underlying the effect of natural antioxidants targeting free



radicals that cause oxidative stress in EAC are explained. By discussing the limitations of natural antioxidant treatment in EAC, the effectiveness of antioxidants in carcinoma is evaluated. The Effect of Antioxidant Property of Flavonoids and Other Plant Extracts on EAC Some vitamins and hormones that perform various functions in the cell are used in the literature to reduce cellular damage due to oxidative stress with their antioxidant properties (Figure 1).

For example, melatonin hormone and its metabolites are known to have antioxidant properties (10). Melatonin, a hormone synthesized and secreted mainly by the pineal gland is a pharmacologically valuable compound with immense cancer chemopreventive potential (11, 12). Although the physiological effects of melatonin vary in the cell, this hormone is involved in detoxification of free radicals, bone formation and protection, and regulation of cardiovascular functions (13).

The antioxidants anticancer effect of apoptotic process by affecting the expression

In the context of its antioxidant effects, the effectiveness of melatonin is also being studied in tumor modeling. In a study conducted in this context, the antitumor activity of melatonin on EAC cells immunized into female BALB / c mice was investigated. It has been reported that the administration of melatonin treatment (20 mg / kg) to mice significantly reduces tumor cell size and neoplastic cell number. In addition, a decrease in anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) level and a significant increase in caspase-9 and caspase-3 protein levels were detected in mice with melatonin treatment. Cancer cells have evolved different mechanisms to evade apoptotic cell death and melatonin has experimentally verified ability to induce apoptotic pathways. It is supported by previous studies that administration of melatonin with different chemotherapeutic agents has beneficial effects on patients with cancer (14). This activity of melatonin in cancer cells is thought to occur by stimulating the production of reactive oxygen species (ROS) and suppressing the tumor antioxidant system and / or the receptor-mediated pathways (15). Melatonin-mediated ROS production refers to the mitochondrial pathway of apoptosis through a mechanism linked to melatonin receptor 1 (MT1) and melatonin receptor 2 (MT2) receptors (16,17). In this context, understanding the MT1 and MT2 receptors involved in melatonin's

suppression of oxidative stress may be important in the development of melatonin as a therapeutic approach (18). The information presented suggests melatonin as a natural chemotherapeutic adjuvant in cancer treatment, tested in clinical trials (19).

The efficiency of antioxidant vitamins in EAC modeling is important. In a study conducted in this context, it was reported that vitamins C, E and pequi oil had positive effects on mice carrying EAC. Antioxidant administration before tumor inoculation efficiently inhibits EAC cell growth. On the contrary, it is stated that after the emergence of the tumor, this practice accelerates tumor growth and directs metastasis. Pequi oil inhibited the growth of the tumor, however, co-treatment with vitamin E and vitamin C promoted metastasis. In this context, apart from vitamin E administration, the development of acid tumor metastasis might be associated with significant increase in the inflammation. Collectively, these findings indicate that efficacy and applicability of antioxidants in the literature not only depends on the nature and type of the antioxidant, the stage and type of cancer being treated, but also on the type of antioxidant therapy (20). In addition, vitamin C is an essential antioxidant in extracellular fluids (21), and in this context, substantial decrease in DNA damage was reported only for antioxidant controls and vitamin C administered before tumor implantation, while vitamin E and pequi oil did not effectively reduce this damage (20). In addition, ascorbic acid has been shown to play a fundamental role in the maintenance of the vasculature and its depletion restricted angiogenesis and retarded tumor growth in mice (22, 23). Importantly, discordance in these findings may be due to variability in the experimental procedures and the type of antioxidants used in wide variety of studies (21, 24).

Flavonoids, one of the natural substances with variable phenolic structure, are found especially in fruits, vegetables, grains and tea. Flavonoids are highly preferred in the development of therapeutic approaches to cancer with their anti-oxidative, anti-inflammatory and anti-carcinogenic properties (25, 26). Some compounds from the flavonoid family, which are currently being studied in the literature, are evaluated in EAC. In a study, in vivo efficacy of leaf and shell extracts of *Morus latifolia* (*M. latifolia*) in EAC was investigated. Leaf and bark extract decreased Bcl-2 expression and increased the expression of Bcl-2-related X (Bax) protein, depending on the dose. Leaf and shell samples of *M. latifolia* inhibit EAC cell growth through the internal apoptotic process by affecting the expression of Bcl-2 and Bax genes. In this regard, *M. latifolia* plant extracts have been shown to contain several acyl compounds that inhibit the growth of cancer cells (27). In another study, the antiproliferative effect of *Basella alba* (*B. alba*), a widely consumed vegetable rich in flavonoid content, together with the molecular signaling of apoptosis in the EAC cell line is evaluated. In this context, it has been stated that in the EAC cell line, *B. alba* has a cell growth suppression character. It is also stated that *B. alba* induces apoptosis in the EAC cell line by up-regulation of the tumor suppressor gene P53 and down-regulation of the antiapoptotic gene Bcl-2. In this context, it has been suggested that leaf and seed extracts of *B. alba* markedly reduced proliferation ability of EAC cell

line (28).

The antioxidant and anti-cancer properties of *Scindapsus officinalis*, which is rich in flavonoid content, made it necessary to evaluate it in the EAC model. In a study conducted in this context, the efficacy of *Scindapsus officinalis* was examined by injecting EAC into Swiss albino mice. Preliminary studies evaluating the anticancer potential of Methanol extract of *Scindapsus officinalis* (MESO) in EAC-bearing mice reveal the presence of plant components such as flavonoids, tannins and glycosides. The presented findings show an increase in survival in mice treated with MESO. In this context, it is stated that the methanol extract of *Scindapsus officinalis* may have an antitumor effect against EAC (29).

The effect of antioxidants on the biochemical mechanism

Brucine (BRU), a natural plant alkaloid, is known to have cytotoxic and antiproliferative activities. In a study conducted in this context, the antitumor activity of BRU, which has antioxidant activity, was investigated in the EAC model. EAC cells were injected intraperitoneally (i.p.) into Swiss albino mice and administered BRU treatment, resulting in time and dose-dependent inhibition of EAC *in vivo*. However, with the course of anti-angiogenic effects, vascular endothelial growth factor (VEGF) and tumor necrosis factor alpha (TNF- α) decrease; Expression of interleukin 12 (IL-12) increased. In this context, BRU reduces microvascular density (30). Besides its antitumor and apoptosis properties, BRU is known to downregulate VEGF expression, an important angiogenic factor for angiogenesis (30). It shows a significant increase in IL-12 production, indicating an elevation of the tumor-reactive microenvironment in mice treated with BRU. The information presented indicates that BRU, which has antioxidant activity, may delay cancer progression by reducing the relationship between inflammation reactions and cancer (30). Another study investigated the antitumor activity of caffeic acid (CA), a natural antioxidant, in mice injected with EAC cells. It has been stated that while CA increases the cytotoxic effects of M1 macrophages, it can inhibit tumor growth. Based on the presented findings, it is thought that the antitumor activity of CA is the result of the stimulation of different mechanisms that affect proliferation, angiogenesis, immunomodulation and survival. CA administration significantly suppresses tumor formation in the EAC model by effectively blocking the formation of tumor-associated macrophages (TAM). In this context, targeting TAMs with antioxidants could be a potentially effective method for cancer treatment (31).

Methanol extract of *Indigofera linnaei* (MEIL) treatment, which has antioxidant activity in the literature, is a compound known to increase the life span of animals by reducing the volume of the tumor. In this context, the efficacy of MEIL extract against EAC cell lines was investigated in a study. MEIL significantly reduced the solid tumor volume *in vivo*. This plant extract also leads to the accumulation of acid fluid in the peritoneal cavity of tumor-bearing animals. The results presented indicate that MEIL demonstrates antitumor activity on EAC tumor cells. At doses of 200 and 400 mg / kg, MEIL

significantly increases the average survival time, but offers a protective effect on the hemopoietic system. These results demonstrate the significant antitumor effect of MEIL against EAC cancer cell lines (32).

In a study, methanol extracts of *Careya arborea* shell (MECA) were tested for hepatoprotective and antioxidant activity in EAC tumor-bearing mice. Various such compounds presented are known to have potent antitumor, hepatoprotective and antioxidant properties (33, 34). In this context, extract treatment at doses of 50, 100 and 200 mg / kg body weight administered orally has been reported to normalize biochemical changes in serum, liver and kidney in animals as hepatoprotective (35). Significant regeneration of all biochemical parameters presented on MECA therapy at all doses tested in the study towards normal values indicates that vital organs are protected from damage caused by EAC. However, similar results have been reported in previous studies in the literature on MECA (36). Therefore, the powerful hepatoprotective and antioxidant properties of MECA is very important in cancer treatment.

Ursolic Acid (UA) is a triterpene that occurs naturally in many plant foods and has antioxidant activity. In a study conducted in this context, the anticancer activity of UA was investigated in EAC. Ursolic acid effectively inhibited tumor growth in mice intraperitoneally (ascitic tumor) and subcutaneously (solid tumor) injected with EAC cells. Histopathological examination of the tumor mass gave indications about chromatin condensation and cell shrinkage. UA treatment downregulates VEGF expression, while Bax and caspase-3 expressions upregulate the pro-apoptotic Bcl-2 family. Ursolic acid caused significant shrinkage of the acid and solid tumor burden in mice without any adverse effects and also increased the survival of tumor-bearing mice. It is stated that UA therapy inhibits EAC cell growth and proliferation *in vivo* with a decrease in acid volume and cell number (37). Since this anticancer agent directly lyses cells by the cytotoxic mechanism, it may indicate that UA has a direct relationship with tumor cells (38). These findings highlighted potent antitumor properties of UA as a promising bioactive product for cancer treatment (37).

In another study, it was reported that mistletoe extract developed a protective effect in female Swiss mice injected with EAC, and oxidative changes in EAC cells caused a decrease in catalase activity and an increase in xanthine oxidase and peroxidase activities (39).

Quercetin is a plant flavonol from the flavonoid group of polyphenols. It is found in many fruits, vegetables, leaves, seeds and grains; Many pharmacological properties of quercetin have been extensively investigated and have been reported to block the progression of various human tumors, such as oral cancer and breast cancer (40). The anticarcinogenic effect of the Quercetin, which contains enough flavonoid substances in its composition as an antioxidant substance, on the EAC cells was evaluated by the silver stained nuclear area / Argyophilic nucleolar region (AgNOR) staining method. They reported that the Quercetin-treated groups decreased the Total AgNOR area / core area value and the AgNOR number compared to the untreated group. As a result, it has been reported to have an antitumoral effect (41).

Conclusion

Traditional treatments such as natural products used in cancer treatment have attracted the attention of the scientific and medical circles, because of their less side effects and costs. Despite significant advances in the research and development of various cancer prevention drugs in the literature, there are two main limitations for the use of existing chemotherapeutic agents. Conventional chemotherapeutic agents are not selective for cancer tissues that cause undesirable side effects in chemotherapy. In addition, the formation of drug-resistant cancer cells with chemotherapy also limits the effectiveness of success. Side effects of antitumor drugs can be reduced by various agents that can distinguish tumor cells from normal proliferative cells. It is aimed to minimize resistance in tumor cells by using a combined approach with different mechanisms of action. At this point, the use of natural antioxidants is thought to be of great importance in the treatment of cancer. In-depth investigation of the effectiveness of antioxidants in cancer treatments has begun to gain momentum, and these increasing studies will also increase collaborative studies across disciplines.

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