Journal of Biomedical Sciences
Research Article Peer-review, Open Access

The Redox Status in Paraneoplastic Disorders and the Impact of Antioxidant Therapy

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Abstract

This paraneoplastic alterations of the redox-status in cancer-bearing laboratory mice and effects of α-tocopherol as a potential treatment strategy have been studied in cancer-bearing mice with the use of electron paramagnetic resonance spectroscopy (EPR). Results of investigation indicate a disruption of iron metabolism, with an accumulation of harmful Fe^{2+} species and oxidative stress markers, such as Met-Hb and Mn^{2+}. The detection of NO and its derivatives indicate ongoing lipid peroxidation and cellular damage, further exacerbated by altered antioxidant enzyme function. Treatment with α-tocopherol provided considerable restoration of redox balance, offering potential therapeutic benefits in reducing oxidative stress during malignancy. The study showed that α-tocopherol increases antioxidant system activity, enhances electron transport at the NADH-ubiquinone oxidoreductase locus, improves mitochondrial respiration, decreases lipid peroxidation and stabilizes membrane structures. **Conclusion:** The findings suggest that α-tocopherol could serve as a potential therapeutic agent to diminish oxidative damage, restore redox balance, and improve microhemocirculation. Further research is needed to explore the implications of these findings for the management of paraneoplastic syndromes and the overall treatment of cancer.

Key words: cancer, paraneoplasia, redox status, blood, liver, α-tocopherol.

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Introduction.

Cancer remains one of the leading causes of death worldwide, with over 19.3 million new cases and nearly 10 million cancer-related deaths in 2020 alone [1]. Beyond the direct effects of tumor growth, cancer can also cause a range of systemic effects, including metabolic disturbances, immune dysregulation, and organ dysfunction. Among these systemic manifestations are paraneoplastic syndromes or disorders [2-4]. Paraneoplastic syndromes occur when cancer cells produce substances that affect distant tissues. These syndromes are not due to the local presence of tumor cells but arise from the systemic effects of malignancy. Paraneoplastic disorders can involve the nervous system, endocrine organs, skin, and other tissues, and they often precede the diagnosis of cancer or arise during its progression [2, 3]. Their effects can significantly impact patient morbidity and mortality, making it essential to understand the underlying mechanisms to improve therapeutic strategies. Effective treatment of these syndromes can enhance quality of life and improve cancer therapy outcomes [4].

One of the key mechanisms involved in both cancer progression and paraneoplastic syndromes is redox imbalance**.** The term redox status refers to the balance between the production of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, and the antioxidant systems that neutralize these reactive molecules. In healthy cells, this balance is tightly regulated to prevent oxidative stress, which can damage proteins, lipids, and DNA. However, in cancer, this redox equilibrium is disrupted. Cancer cells often exhibit elevated ROS levels due to increased metabolic activity and mitochondrial dysfunction, leading to oxidative stress [5-7]. Oxidative stress plays a dual role in cancer. On one hand, it promotes DNA mutations, genetic instability, and tumor growth, while on the other, excessive ROS levels can induce cell death. Thus, cancer cells develop adaptive mechanisms to manage oxidative stress, including the upregulation of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase. However, the disruption of redox homeostasis can also contribute to the development of paraneoplastic syndromes, particularly through the damage to red blood cells (RBCs) and other cellular membranes, leading to impaired microcirculation, hypoxia and organ malfunctioning [8-10].

Considering the pivotal role of oxidative stress in cancer and its systemic effects, research into antioxidant therapies has gained increasing attention. Agents such as α-tocopherol (vitamin E), a powerful lipid-soluble antioxidant, have been investigated for their potential to restore redox balance, stabilize cell membranes, and improve mitochondrial function [11].

Among numerous paraneoplastic syndromes disorders of microhemocirculation must be emphasized especially. It is very common for malignant growth and could be considered as one of the reasons of poor outcome at routine care of cancer patients. Intact microhemocirculation to a great extent depends on the RBCs properties. RBCs are very labile, fragile, immediately reflecting any deviations occurring in organism. RBCs functional state, closely related with their membrane deformability has power to alter hemorrheology and intensity of microcirculation [12, 13]. Disorders of RBCs deformability and microcirculation in turn are responsible for generalized hypoxia accompanying malignant tumor growth supporting disease progression. Disorders of microhemocirculation alter redox status leading to further undesirable events, or so called “vicious circle” leading to cancer progression [14-17].

We aimed to investigate electronic-paramagnetic centers in the blood and liver to evaluate redox status during cancer growth, assess the effects of α-tocopherol as a free radical scavenger, identify mechanisms underlying paraneoplastic disorders of RBC membrane and their role in microhemocirculation disorders. We suppose that understanding paraneoplastic disorders of redox status in an "intact" cells and tissues would help to detect appropriate treatments for their correction, which could significantly increase the efficacy of conventional therapies. Understanding the interplay between cancer, redox status, and paraneoplastic disorders may provide new avenues for therapeutic interventions aimed at improving patient outcomes and quality of life.

Material and methods

Experiments were conducted on BALB/c lab mice with the 20-25 g. body mass. All animals were fed standard laboratory chow and given free access to water. The care and use of the animals complied with the Georgian regulations on protection of animals, with Guidelines prepared by the Ethics Committee of the Institutional Animal Care and with the National Institutes of Health Guide for the Care and Use of Laboratory animals.

Experimental animals were randomly divided into three major groups: The Group I involved healthy mice, the Group II was control (Ehrlich carcinoma bearing, untreated mice) and the Group III was experimental (mice treated with α-tocopherol).

Electronic-paramagnetic signals of the blood and liver were studied with the use of electronic-paramagnetic resonance (EPR) method on $17^{th}$and 24^{th} days of Ehrlich carcinoma growth. The blood and liver samples were placed in polyethylene tubes and kept in liquid nitrogen (-196^0C). EPR specters were registered on the radiospectrometer РЭ-1307 (Microwave radiation – 20 mVt) at room temperature. Free nitric oxide (NO) in the blood has been defined using the spin-trap –Na-diethyldithiocarbamat (DETC) (SIGMA).

Intraperitoneal injections of α-tocopherol (1,2 ml/100 g a day, during 10 days, beginning from the 7^{th} day after $1x10^6$ Ehrlich carcinoma cell inoculation, when the average volume of cancer tissue was 0,7 cm^3) were used as an antioxidant and membrane stabilizing agent.

Obtained data were analyzed statistically with the use of SPSS 16.0 for Windows. Differences between tumor bearing control (untreated) and treated animals were determined by using the Student's *t* test. The criterion for significance was set to $p < 0.05$.

Results and discussion

The investigation results showed that in Ehrlich carcinoma-bearing mice, the intensity of EPR signal of nitric NO and oxidized form of ceruloplasmin (by detecting the paramagnetic Cu^{2+} ions) in the blood increased, while the EPR signal of Fe^{3+}-transferrin decreased. During the same period of malignant growth, the EPR spectrum of the blood showed the methemoglobin (Met-Hb), Fe^{2+}, and Mn^{2+} signals.

The shift in iron metabolism and redox balance observed in Ehrlich carcinoma-bearing mice, indicated by decreased Fe^{3+}-transferrin and the detection of Met-Hb, Fe^{2+}, and Mn^{2+}, reflects significant oxidative stress and impaired metal homeostasis. This disruption in iron regulation has major downstream effects on essential biological processes, including hemoglobin synthesis, mitochondrial respiration, and overall energy metabolism.

Iron Metabolism and Fe^{3+}-transferrin

Fe^{3+}-transferrin is crucial for transporting iron to tissues where it is used for hemoglobin synthesis, cellular respiration, and various enzymatic reactions. A decrease in Fe^{3+}-transferrin suggests that iron is not being adequately transported, which could lead to iron accumulation in its ferrous (Fe^{2+}) form. This accumulation is harmful, as Fe^{2+} is highly reactive and can participate in the Fenton reaction, generating reactive oxygen species that damage cellular components [18]. Insufficient iron delivery affects hemoglobin synthesis, mitochondrial function, and overall cellular metabolism, potentially leading to anemia and reduced oxygen transport in the body. Additionally, the disruption of iron metabolism in cancer is linked to increased tumor progression and aggressiveness, as tumors often manipulate iron availability for their growth [19].

Methemoglobin (Met-Hb) and Hemolysis

Met-Hb forms when hemoglobin's iron is oxidized from its ferrous (Fe^{2+}) to ferric (Fe^{3+}) state, rendering it unable to bind oxygen effectively. In normal physiological conditions, methemoglobin reductase (such as NADH-cytochrome b5 reductase) converts Met-Hb back to functional hemoglobin. However, during

oxidative stress, the capacity of this reductive system is overwhelmed, leading to Met-Hb accumulation [20, 21]. Elevated levels detected through EPR spectroscopy are a direct indicator of hemolysis, a condition where oxidative stress damages red RBCs causing them to rupture and hemolysis.

Mn^{2+} and Antioxidant Defense Dysfunction

The signal of Mn^{2+} in EPR spectra suggests the involvement of manganese in the redox balance, particularly as a cofactor for manganese superoxide dismutase (Mn-SOD), an antioxidant enzyme crucial for detoxifying superoxide radicals [22]. During oxidative stress, increased Mn^{2+} levels reflect enhance in Mn-SOD activity to reduce ROS damage. However, excess Mn^{2+} can also inhibit Mn-SOD activity, leading to exacerbated oxidative damage, particularly in mitochondrial membranes [23]. The increased signal of Mn^{2+} in the EPR spectrum suggests that manganese-dependent antioxidant systems are overwhelmed, contributing to the overall oxidative stress. The role of Mn^{2+} in lipid peroxidation and mitochondrial dysfunction is critical during cancer progression, where oxidative stress is a consequence and a driver of malignancy.

Fe^{2+} and Peroxidative Processes

Fe^{2+} is a powerful generator of free radicals and an activator of peroxidative processes. Presence of Fe^{2+} EPR signal indicates that there is a higher level of unbound ferrous iron in the system. Fe^{2+} is the reduced form of iron and under normal physiological conditions, is carefully controlled due to its ability to generate harmful reactive oxygen species via the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {\bullet}OH + OH^-$). Free Fe^{2+} can catalyze the formation of hydroxyl radicals (•OH), the most reactive and damaging ROS, leading to cellular damage (oxidative damage of proteins, lipids, and DNA).

The combination of decreased Fe^{3+}-transferrin and increased Fe^{2+}, along with the Met-Hb and Mn^{2+}, reflects a significant disruption in iron metabolism and redox homeostasis in carcinoma-bearing mice [24].

Ceruloplasmin and Reactive Oxygen Species

Ceruloplasmin acts as a Fe^{2+} scavenger. Its main enzymatic function is to oxidize Fe^{2+} (ferrous iron) to Fe^{3+} (ferric iron), facilitating the binding of iron to transferrin for transport. Increased activity of EPR signals of oxidized ceruloplasmin and Fe^{2+} ions observed in our study indicates a decrease in antioxidant system activity, thereby increasing the potential for cellular injury. This process supports the formation of free radicals, amplifying lipid peroxidation (LPO) in biological membranes. Oxidative stress produce imbalance between the production of ROS and the body's ability to detoxify them. Since ceruloplasmin acts as an antioxidant by scavenging superoxide radicals, an increase in its oxidized form suggests that the body may be responding to elevated levels of oxidative stress. An increase in the oxidized form of ceruloplasmin might reflect the body's attempt to cope with an excess of ROS, as it works to maintain redox balance [25].

Increased EPR signals of oxidized ceruloplasmin and Fe^{2+} ions, along with decreased EPR signals of Fe^{3+}-transferrin, negatively affect erythropoiesis. The intense LPO and subsequent membrane damage are further exacerbated by Mn^{2+}-containing complexes. These complexes inhibit the antioxidant enzyme superoxide dismutase. Thus, during cancer growth, the increased presence of oxidized ceruloplasmin, Mn^{2+} complexes and Fe^{2+} in the EPR spectra of the blood plays a key role in the progression of LPO.

Nitric oxide (NO), Peroxynitrite, and Oxidative Stress

Disruptions in membrane structure, including RBC membranes, are also promoted by NO, another key driver of peroxidation processes, as revealed in the EPR spectrum of the blood. NO, while a normal signaling molecule, becomes pathological in the presence of ROS, particularly when it reacts with superoxide radicals to form peroxynitrite ($ONOO^-$), a highly reactive and damaging species. Under the hypoxic conditions inducible nitric oxide synthase (iNOS) is activated, driving the excessive production of NO. The interaction of NO with free radicals exacerbates oxidative stress and lipid peroxidation,

leading to structural damage in cellular membranes, including those of RBCs. Peroxynitrite, along with other ROS, plays a key role in the oxidation of iron in hemoglobin, contributing to the formation of Met-Hb and promoting hemolysis [20, 21].

The exaggerated production of NO is likely due to disordered local hemocirculation and microcirculation. Under generalized hypoxia accompanying malignant tumor growth, nuclear factor NFkB activates iNOS, which leads to increased NO production. NO, when combined with free radicals, forms highly reactive peroxynitrite, leading to progressive cell membrane damage . Thus, the excessive production of POL promoters (Fe^{2+}, Mn^{2+} and NO), results in the destruction of cell membranes and hemolysis. A strong indicator of hemolysis is the presence of intense EPR signals of Met-Hb, which are not typically detected under normal conditions [26, 27].

Liver Redox Imbalance

EPR signals of the liver are primarily associated with the presence of paramagnetic species involved in key metabolic and redox processes. The liver plays a central role in detoxification, iron and copper storage, and redox regulation. EPR analysis of the liver revealed increased signals of free radicals and iron-sulfur (Fe-S) clusters, indicating mitochondrial dysfunction, especially in the cytochrome P-450 locus. Damage to microsomal membranes compromises electron transport processes, affecting detoxification pathways and the overall efficiency of the liver's monooxygenase system [28, 29].

Disruption or damage to the microsomal membranes can negatively impact the function of the monooxygenase system in several ways: i) The cytochrome P-450 enzymes, which are integral to the microsomal membranes, rely on the proper membrane structure and environment to function correctly. If the membranes are damaged, the activity of these enzymes can be reduced or dysfunctional. Lipid peroxidation of the membranes caused by oxidative stress can further alter the fluidity and composition of the membranes, affecting the functioning of membrane-bound proteins, including cytochrome P-450 enzymes, ii) The cytochrome P-450 enzymes require electron transport from NADPH via NADPH-cytochrome P-450 reductase to carry out the oxidation reactions necessary for detoxification. If the microsomal membranes are compromised, the electron transport processes may be disrupted, reducing the efficiency of the monooxygenase system. End result is disorders of detoxification processes in the liver**.** The appearance of nitrosyl complexes of non-heme iron indicates the presence of hypoxia, aligning with the elevated Met-Hb levels observed in the blood [30].

α-Tocopherol's Therapeutic Impact

Following treatment with α-tocopherol, a considerable reduction in EPR signals of Fe^{2+} and Mn^{2+} was noted, with the restoration of Fe^{3+}-transferrin activity. EPR signals of oxidized ceruloplasmin, Met-Hb, NO, and Fe-S-nitrosyl complexes decreased significantly. This reflects an overall improvement in redox status, characterized by reduced oxidative stress and enhanced iron regulation. The diminished intensity of free radical signals suggests a stabilization of redox processes and a reduction in oxidative damage.

The antioxidant properties of α-tocopherol act to stabilize membranes, prevent lipid peroxidation, and maintain the structural integrity of RBCs, thus enhancing their deformability and microcirculation. The reduction of oxidative stress-related products such as Met-Hb and the normalization of iron levels through α-tocopherol administration suggest that this treatment can help restore redox balance and improve the overall functionality of RBCs.

The restoration of these parameters could lead to better oxygen delivery to tissues, ultimately reducing the hypoxia associated with paraneoplastic syndromes. Moreover, the improved RBC deformability resulting from α-tocopherol treatment suggests that antioxidant therapy may provide benefits in maintaining microcirculation and overall organ function in cancer patients.

Conclusion.

Cancer cells generate excessive ROS which leads to oxidative stress. Enhanced LPO in the membranes of RBCs and other blood components is common, leading to membrane instability and cell damage.

Antioxidant enzymes are overwhelmed by the increased production of ROS, reducing their effectiveness in neutralizing oxidative damage.

Elevated NO levels in the blood, often due to increased activity of iNOS, can lead to the formation of peroxynitrite, a highly reactive molecule that damages cell membranes and proteins.

Hemoglobin undergo oxidation, leading to the formation of MetHb, contributing to hypoxia and further oxidative stress.

Mitochondrial dysfunction with disrupted electron transport chain activity, particularly at the NADH-ubiquinone oxidoreductase locus leads to an increased production of ROS. The presence of increased signals of $Fe^{2+}$and Mn^{2+}in liver cells promotes further oxidative stress by catalyzing the formation of ROS through the Fenton reaction, leading to further progressive peroxidation of lipids and cellular damage.

The liver, being a major site of detoxification, is particularly vulnerable to oxidative stress. Elevated LPO in the liver's cellular membranes lead to hepatocyte damage and impaired liver function. The liver's antioxidant defenses are compromised. Enzymes such as glutathione peroxidase and catalase are less effective, while levels of glutathione, a critical antioxidant, are depleted. This exacerbates oxidative damage in liver tissues and affects the liver's ability to metabolize and excrete toxins, detoxify harmful substances. The detoxification processes in liver is altered also due to disorders in microsomal membranes that impair functioning of the monooxygenase system, especially the cytochrome P-450 enzymes**.**

Targeted antioxidant therapy helps restore redox balance by scavenging free radicals and reducing LPO in both RBCs and hepatocytes.

By enhancing the activity of antioxidant enzymes and improving electron transport at the NADH-ubiquinone-oxidoreductase locus, α-tocopherol mitigates mitochondrial dysfunction and supports normal energy production. α-tocopherol protects the integrity of cell membranes in the RBCs and liver, reducing oxidative damage thereby supporting detoxification functions of the liver, preventing further hemolysis, disorders of microcirculation and hypoxia.

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