



OXIDATIVE STRESS AND ANTIOXIDANTS IN BRAIN TUMORS

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Abstract. The brain has a particular predisposition to oxidative stress which makes it vulnerable to free radicals. They trigger lipid peroxidation of the cellular membranes, oxidation of proteins and DNA leading to changes in chromosome structure, genetic mutation, and/or modulation of cell growth. It was shown that oxygen-derived free radicals play an important role in brain tumor development due to DNA strand breaking, appearance of point mutations and aberrant DNA cross-linking. Consequently, a genomic instability is created which contributes to carcinogenesis. In the next steps of carcinogenesis, oxygen free radicals may initiate signal transduction pathways and activate fundamental transcription factors such as NF-kB, Nrf2 or HIF-1. In this context, the contribution of oxidative stress in brain tumors genesis and the role of antioxidants were analysed.

Keywords: oxidative stress, glioma, meningioma, antioxidant

Introduction

Brain tumorigenesis is today associated with oxidative stress. This is reflected by an imbalance between free radicals production and antioxidant mechanisms. In pathological conditions, free radicals are generated in excess from endogenous sources (mitochondria, peroxisomes, inflammatory cell activation, neurotransmitters oxidation) and exogenous sources, including environmental agents, drugs, irradiation, chemicals. The resulted oxidative and nitrative stress promotes various pathologic reactions which contribute to neurodegenerative disorders, atherosclerosis, chronic inflammation [1]. Other studies have suggested that chronic oxidative stress, particularly from chronic inflammation, is associated with diabetes and cancer [2].

In response to various inducers, large amounts of free radicals (superoxide anion - O_2^- , hydrogen peroxide $-H_2O_2$ and hydroxyl $HO\cdot$) trigger lipid

peroxidation of the cellular membranes, oxidation of proteins and DNA and lead to changes in chromosome structure, genetic mutation, and/or modulation of cell growth. It was shown that oxygen-derived free radicals play an important role in tumor development. The induction of cancer represents a multistage, multistep process, involving multiple molecular and cellular events. During the transformation of a normal cell into a malignant one, initiation, promotion and progression stages were described [3].

Among factors well established in oxidative stress related carcinogens, ionizing radiation can function at all stages of carcinogenesis. They induce cancer in several species, in almost all organs examined [4]. Ionizing radiation produces the radiolysis of water, generates free oxygen radicals and alters key molecules which can persist and produce a long term influence on target cells, generating delayed brain tumors [5].

Chemical compounds that induce oxidative stress and damage in vitro and in vivo include chlorinated substances, metal ions, phorbol esters. It was demonstrated that acrylonitrile induced oxidative stress in rat brain tissue and cultured rat astrocytes. This oxidative damage characterized by lipid peroxida-

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tion, oxidative DNA damage and the modulation of antioxidant defense produced brain tumors in rats.

Oxidative stress may also influence gene expression. H_2O_2 activates a large group of signaling kinases (protein kinases, phosphoinositide 3-kinase/serine-threonine kinase, protein kinase B and protein tyrosine phosphatases) involved in the regulation of cell migration, proliferation, survival, and apoptosis. An abnormal modulation of these genes may be the gate for oxidative stress induced carcinogenesis.

Nuclear factor erythroid 2-related factor 2 (Nrf2) represents a transcription factor which regulates the expression of many antioxidant enzymes (SOD, CAT, GTS, GPX). Its role is still controversial taking into account that low levels of Nrf2 or loss of Nrf2 predispose cells to tumorigenesis, but instead, elevated activity of Nrf2 may favor evolution of cancer by offering chemoresistance to antitumoral treatment [6].

Nuclear factor- κ B is the name for a family of transcription factors. The NF- κ B

regulate the inflammatory gene expression, the genes which encode proinflammatory cytokines (TNF α , IL-1 β , and IL-12), cell adhesion molecules (vascular cell adhesion molecule-1 and intercellular cell adhesion molecule-1), iNOS, and COX-2. These molecules, together with NO derived from iNOS and COX-2-produced PGE₂, play important roles in the pathogenesis of inflammation and neurodegenerative disease, but also of cancer genesis and progression. There are evidences for a role of NF- κ B in the proliferation and survival of glioblastoma cell lines. It was reported that NF- κ B activation in response to chemotherapeutic agents protected some glioblastoma cells lines in vitro. Data showed a correlation of NF- κ B activity with tumor grade. NF- κ B activity represents not only a signal that glioma cells are active, but its activity increases progressively with malignancy [7].

Nitric oxide (NO) is one type of reactive nitrogen species. Nitric oxide is synthesized from L-arginine by the NO synthase (NOS). Three isoforms of the NOS have been described. Two isoforms are expressed constitutively (cNOS), the neuronal (nNOS or type 1) and the endothelial (eNOS or type 3), and one inducible under pathological conditions (iNOS or type 2). In gliomas was described the upregulation of NOS, with increased NO synthesis and peroxynitrite (OONO⁻) formation. OONO⁻ breaks DNA strand, induces point mutations and aberrant DNA cross-linking and create a genomic instability which contributes to carcinogenesis [8].

Brain tumor development involves not only oxidative aggression but also a reduced response of antioxidant defense. During prolonged oxidative stress, changes in brain antioxidant enzymes

activities, including superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione transferases (GTSs) appear. These enzymes normally act to prevent or decrease brain damages caused by free radicals in excess. However, they are controlled by polymorphic genes which can be altered by free radicals, leading to dysfunctions in enzymes' activity. Their role in brain tumors genesis is sustained by several studies.

Rao showed that the red-blood-cell activity levels of SOD were decreased for most types of intracranial neoplasm [9].

GPX₁ is the most abundant selenoprotein in mammals; it is ubiquitously expressed in humans and protects cells against oxidative damage by reducing hydrogen peroxide and other of organic peroxides [10]. It was shown that in patients with brain tumors (meningioma and glioblastoma) after lead exposure, the variant GPX₁ enzyme is less responsive to stimulation and may promote the development of cancer because of reduced levels of protection against oxidative damage from reactive oxygen species. Enzyme activity was also decreased by lead binding to the enzyme active center and may also result in reduced levels of protection against oxidative damage [11, 12].

Glutathione S-transferases (GSTs) represent II phase reactants which detoxify free radicals into water-soluble chemicals, through conjugation. During this process, the activity of these enzymes may be altered and modify the risk of cancer. It was found that GSTM1 and GSTP1 polymorphisms may play a role in brain tumor susceptibility by histological subtype, especially high-grade pediatric astrocytomas [13].

Catalase (CAT) represents an endogenous antioxidant enzyme from red blood cells which converts H₂O₂ into H₂O and O₂ [14]. During prolonged oxidative stress it may be upregulated. It was shown that the genetic polymorphisms of the CAT gene play a role in ROS-induced carcinogenesis [15]. On the other hand, the impaired role of the immune system for the brain tumors' development was emphasized by Conti, in a relevant review [7]. In the complex process of glioma development, the microglia dysfunctions regarding antigen presentation play a role; the immune dialog between CD4⁺ T cell and CD8⁺ T lymphocyte expressed through IL-2, IL-4, IL-7, IL-12 and INF- γ secretion is altered by immuno-inhibitory products such as transforming growth factor (TGF)- β , IL-10, insulin-like growth factor I, and prostaglandin E₂ (PGE₂), originating in glioma cells. It was shown that the phenomenon of tumor cells immortalization involves antiapoptotic proteins such as Bcl-2, TRAF1, and members of the IAP family of genes (survivin) identified in human astrocytic tumors

but not in normal brain tissue [16].

Despite efforts to improve treatment of brain tumor, survival remains limited. Current therapy consists of a combination of surgery, irradiation and chemotherapy with predisposition to long-term complications. However, hemorrhagic side effects may appear after surgical treatment of these tumors [17, 18].

Because the process of identifying of novel targeted therapies is effervescent in this field, we analysed the utility of antioxidant strategies for brain tumors treatment, strategies addressed both to antioxidants and immunomodulators [19].

Antioxidant enzymes derivatives

Extracellular SOD (SOD3) is expressed in the brain but in substantially lower concentrations than SOD1 or SOD2 [20]. SOD activity decreases with age [21]. As cancer cells are sensitive to oxidative stress, modulation of SOD has been valorified as a mechanism to selectively kill cancer cells. The study of a lipophilic analogue of manganese porphyrin in a preclinical brain tumor model, showed that MnTnHex-2-PyP(5+) increases median survival by 33% in adult glioblastoma multiforme (D-256 MG; $p \leq 0.001$) and 173% in pediatric medulloblastoma (D-341 MED, <0.001). The compound is a powerful SOD mimic, a peroxynitrite scavenger and a potent modulator of redox-based cellular transcriptional activity. The use of Mn porphyrin-based SOD mimics, is a promising approach for brain tumor therapy [22].

A recent study demonstrated a new biological property of NADH and proposed NADH for treating gliomas, due to significantly decreased glioma cell survival via oxidative stress and PARP activation [23].

A similar role was recently described for NADPH (nicotinamide adenine dinucleotide phosphate, reduced form). Authors found that the treatment of C6 glioma cells with NADPH induced a significant decrease in the survival of the glioma cells, but had no effect on the survival of primary astrocyte cultures. The results sustained that NADPH decreases glioma cell survival by inducing the NADPH oxidase-dependent increase in oxidative stress and by PARP activation, suggesting a potential therapeutic effect of NADPH on gliomas [24].

Lazaroids

Several lipid peroxidation inhibitors have been tested. The class named lazarooids (21-aminosteroids) were derived from glucocorticosteroids, without glucocorticoid

and mineralocorticoid activities. They removed lipid peroxy radicals and inhibit iron-dependent

lipid peroxidation. The most notable was Tirilazad, a non-glucocorticoid steroid. Despite impressive preclinical evidence that Tirilazad improved outcome through its action, in clinical studies Tirilazad disappointed [25].

COX inhibitors

Joki et al. demonstrated that NS-398, a COX-2-specific inhibitor, increased apoptosis, reduced proliferation, and reduced invasion of cultured human glioma cells [26]. Other recent studies on COX-2 inhibitors also suggest a mechanism involving the suppression of neovascularization and tumor growth [27]. Independent of these results, it was showed that gamma-linolenic acid (GLA) induced a selective glioma apoptosis without affecting normal cells. Comparative to cyclooxygenase (COX) and lipoxygenase (LO) inhibitors, GLA produced a 2-3-fold increase in free radicals and lipid peroxides and decreased the anti-oxidant content of tumor cells. In three clinical studies, intra-tumoral injection of GLA induced significant reduction of glioma without any significant side effects. GLA has a low neurotoxicity and selective activity against tumor cells, that it could be an effective anti-glioma treatment [28].

Iron chelators

A possible role for antiproliferative effects of deferoxamine in vitro and in vivo is based on the observation that the intracellular pool of iron is necessary for DNA synthesis. In a series of clinical studies desferoxamine provided an antitumor activity in the treatment of neuroblastoma, leukemia, bladder carcinoma, and hepatocellular carcinoma [29].

Based on iron's role in brain oxidative stress and tumorigenesis, a recent epidemiological study reported that iron reduction by phlebotomy was associated with decreased cancer risks in a general population. Given that the required amounts of iron decrease during ageing, the fine control of body iron stores would be a wise strategy for chemoprevention of several diseases [30].

Vitamins and vegetal antioxidants

The protective effect of this group is based on their antioxidant properties, enhancement of the immune response, inhibition of mutagenesis and reduction of induced nuclear damage.

Vitamin E is known as one of the most potent antioxidants, by inhibiting the propagation of the free radical chain reaction in the lipids of biological membranes. A group of natural compounds have also been demonstrated to provide promising treatment results. Tocopheryl succinate, a vitamin

E analog, for example, is reported to induce ROS generation and kill cancer cells [31].

β Carotene possesses antioxidant activity analogous to that of vitamin E. Plasma levels of both, carotenoids and vitamin E were significantly reduced in patients with brain tumors and the values were even lower in malignant tumors, when compared to relatively benign tumors [32].

Vitamin C (ascorbic acid)- a water-soluble antioxidant which inhibits peroxidation of membrane phospholipids, acts as a scavenger of free radicals and may also regenerate vitamin E. Brain concentration of vitamin C is 10-fold higher than its plasma levels indicating a potential cerebro-protective role [33].

Vitamin D deficiency during gestation predisposes to brain tumor formation later during life due to long-term effects on brain development [34]. After birth, vitamin D inhibits growth and triggers apoptosis in neuroblastoma and glioma cells [35, 36].

Other data regarding dietary antioxidant and malignant glioma are controversial and vary by histology group. In a cohort of glioma patients, a case-control study found a number of significant inverse associations between antioxidant intake and genesis of glioma. Statistically significant results were found among isoflavones, carotenoids - lycopene, lignans, vitamin C, vitamin E and folate [37].

The natural polyphenol from grapes Resveratrol (3,4',5-trihydroxy-trans-stilbene) had antioxidant, anti-inflammatory and antiproliferative effects on a variety of cancer cells in vitro and in various animal models. It showed cancer chemo-preventive properties and therapeutic potential. In a glioma cells model, resveratrol inhibited cell proliferation and induced cellular senescence. It also inhibited clonogenic efficiencies in vitro and tumor growth by inhibiting the ubiquitin ligase of histone H2B [38].

Resveratrol also strongly inhibits brain tumor cells [39, 40]. It acts synergic with quercetin inducing senescence-like growth arrest and an association was proposed between these compounds to amplify their antitumoral activity for glioma treatment [41]. When acting alone, quercetin enhances glioma cell apoptosis but in the same time provided a selectivity expressed by protection of healthy brain cells [42,43].

Another study evaluated anti-oxidative and apoptosis inducing effect of a polyphenolic compound, ellagic acid. It increases the activity of three antioxidant enzymes, SOD, CAT and GPX and exhibits anti-oxidant activity in a normal cell line and apoptosis induction activity in a cancer cell line, through the down-regulation of Bcl-2/Bax and activation of caspase-3 [44].

A similar role was also described for Zingiber officinale and Chelidonium majus. It was found

that previous extracts could protect against lipid peroxidation, but they are partially effective in alleviation DNA damages [45, 46].

Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine) released by the pineal gland, was shown to exert neuroprotection by inhibiting oxidative stress. New evidences suggested that melatonin may be useful in the primary brain tumors treatment. In physiologic concentrations, melatonin inhibits growth of neuroblastoma cells and was suggested for treating glioma [47].

Conclusion

There are growing evidences suggesting that antioxidants may be useful in treating primary brain tumors. A dynamic relationship exists between oxidative stress and brain tumor appearance and for each case, a multifactorial context should be considered. Tumor microenvironment is a key player in the neoplastic process. The role of antioxidant therapy should be judged not only depending on each type of antioxidant, but also in a time dependent manner. A specific substance may be beneficial in tumor prevention, when it may reduce inflammatory and oxidant aggression, but it may be unfavorable in the presence of an already existent tumor, when a reduction in oxidative stress may interfere with chemotherapy and may produce chemoresistance. Even though many antioxidants proved really encouraging results, further studies in this field are necessary.

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