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Review

Polyunsaturated fatty acids and gliomas: A critical review of experimental, clinical, and epidemiologic data

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ABSTRACT

Certain polyunsaturated fatty acids (PUFAs) called essential fatty acids (EFAs) cannot be biosynthesized by the body and hence, need to be obtained from diet. These PUFAs and their metabolites have multiple physiological functions that are altered in tumor cells due to a decreased expression of Δdelta-6-desaturase, which is an essential step in their metabolism. As a result, tumor cells would be protected from the toxic effect caused by free radicals, one product of EFA metabolism. EFAs have been proposed to have therapeutic potential in the treatment of glioblastoma. Gliomas are the most common primary tumors of the central nervous system in children and adults. High-grade gliomas remain a therapeutic challenge in neuro-oncology because there is no treatment that achieves a significant improvement in survival. Novel therapeutic strategies that use PUFAs for the treatment of gliomas have been assessed in cell cultures, rodent glioma models, and humans, with encouraging results. Here we review the latest progress made in the field.

Essential polyunsaturated fatty acids: Metabolism and function

Lipids are a heterogeneous group of substances widely distributed in animals and plants that play important roles in human biology. Polyunsaturated fatty acids (PUFAs) that contain ≥2 double bonds, and cannot be biosynthesized by the human body are called essential fatty acids (EFAs), and their dietary intake is essential for homeostasis [1]. The terms *PUFAs* and *EFAs* are here used synonymously, although this is not strictly correct.

EFAs include the "ω-3" series derived from α-linolenic acid (ALA, 18:3, ω-3) and the "ω-6" series derived from *cis*-linolenic acid (LA, 18:2, ω-6) [2,3].

Once they are acquired by dietary intake, both LA and ALA are transformed by cellular enzymes, such as desaturases and elongases, into intermediate and final metabolites, which play different physiological roles (Fig. 1). EFAs and their metabolites have been shown to affect tumor cell survival in vitro and in vivo in a wide range of tumors [2,4].

In many tumor cells, EFA metabolism is abnormal because there is a decrease in delta-6-desaturase (D6D) enzymatic activity, which is an essential step for γ-linolenic acid (GLA) formation. However, metabolites derived from the ω-3 series are not affected because they can be obtained from diet [5]. GLA, arachidonic acid (AA), and eicosapentaenoic acid (EPA) possess tumoricidal action due to their ability to produce free radicals that lead to lipid peroxidation [6–8]. Thus, it is believed that a decrease in D6D activity would constitute a protective mechanism used by cancer cells to avoid the cytotoxic effects of EFAs.

It has been suggested that EFAs have therapeutic potential in the treatment of glioblastoma (GB). Factors involved in glioma genesis are multiple and include not only genetic predisposition but also environmental exposure [9]. Dietary nutrients and anti-inflammatory agents that may be involved in glioma risk and

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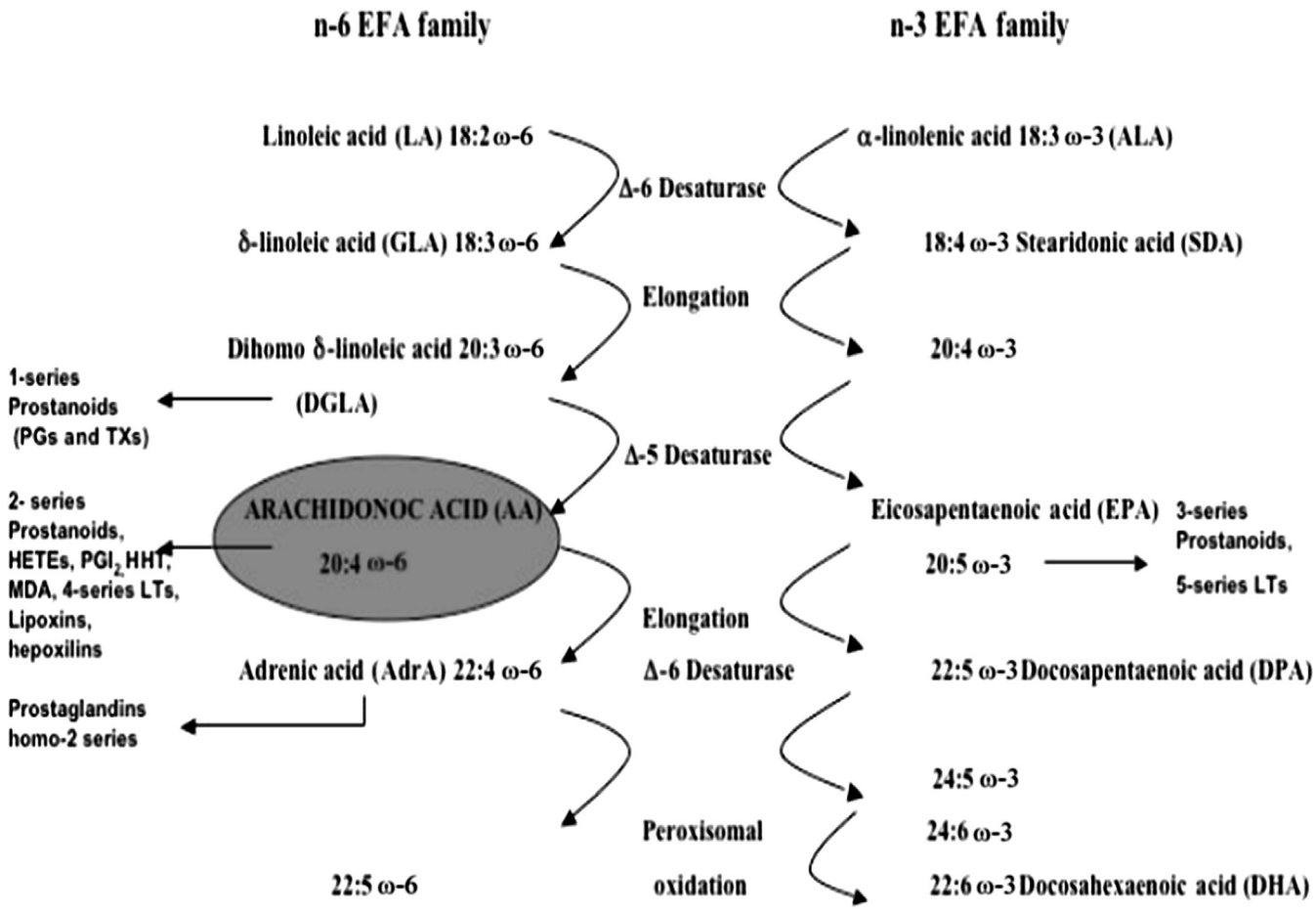

Fig. 1. Cellular pathways of metabolism of ω-3 and ω-6 of polyunsaturated fatty acids. Adapted from Pasqualini ME, Berra MA, Yurawecz MP, Repossi G, Eynard AR. Dietary manipulation of precursor polyunsaturated fatty acids modulates eicosanoid and endocannabinoid synthesis: A potential tool to control tumor development. *Curr Nutr Food Sci* 2008;4:161–75.

progression have been studied. It has been reported that the composition of EFAs differs between glioma tissue and non-neoplastic brain and that these fatty acids exhibit tumoricidal activities without significant side effects. Hence, it has been questioned whether GLA administration may be a suitable adjuvant therapy for the treatment of gliomas or the prevention of recurrences [10].

Gliomas: General features

Gliomas are central nervous system (CNS) tumors of glial origin [10]. The term *glioma* includes the following neoplasms:

1. ependymal tumors, which resemble ependymal cells;
2. astrocytic tumors, which resemble astrocytic cells; and
3. oligodendroglial tumors, which resemble oligodendroglia.

Classification

These tumors are classified according to the 2007 World Health Organization grades, which are a modification of the St. Anne-Mayo grading system scheme [11]. According to this system, tumors with low proliferative potential and with possibility of cure following surgical resection alone are grade I. Grade II lesions are generally infiltrative in nature and often recur. Grade III includes tumors with histologic evidence of malignancy, including nuclear atypia and high mitotic activity. Grade IV is assigned to tumors looking cytologically malignant, mitotically active, with necrosis often associated with bad evolution and fatal outcome [12]. Furthermore, it is considered that GBs (grade IV astrocytomas) can come from two progression pathways, which correlate with two different entities based on their molecular profile. Primary GBs are characterized by a relatively high frequency of epidermal growth factor receptor gene amplification, phosphatase and tensin homolog gene deletion, and cyclin-dependent kinase inhibitor 2 A (p16) loss. Secondary GBs or type 1 GBs, progress from lower grade astrocytic tumors that contain *TP53* mutations [13].

Epidemiologic features

A large epidemiologic study of glial and non-glial brain tumors (N = 45,000) in Europe reported that 86% were astroglial, 6.4% oligodendroglial, and 3.6% ependymal [14].

A recent study estimating the prevalence of primary brain tumors in the United States reported that the overall incidence rate for primary brain tumors was 18.1 per 100 000 person-years. The glioma subgroup was comprised mainly of malignant tumors (96.4%), GBs representing 53.5% of them [15].

In South America, CNS tumors are the most common solid tumors in childhood and GBs are the most common primary brain tumors in adults [16–19].

Taken together, these data indicate that gliomas are the most frequent tumors of the CNS and have an important incidence in the population and a high impact on health systems worldwide because of their high rates of morbid mortality.

Treatment

Patients with low-grade gliomas have a median survival ranging from 5 to 16 y. There is no consensus in the literature regarding the ideal treatment of low-grade gliomas (astrocytoma, oligodendroglioma, and oligoastrocytoma). The use of radiotherapy followed by chemotherapy, preferably temozolomide (an oral alkylating agent), is recommended in tumors when resection is subtotal or gliomas continue to progress. Recent data show that a delay in tumor progression and malignant degeneration was observed with gross total resection of low-grade gliomas. Moreover, an improved overall survival independent of age, degree of disability, and histologic subtype was associated with complete resection [20]. The use of radiotherapy alone in low-grade tumors after surgery is highly questionable because it showed no effect on the overall survival [21].

Median survival of patients with grade III astrocytoma is 2 to 3 y, and for patients with GB it is 12 mo [22]. Surgery followed by 30 fractions of radiation (60 Gy) was suggested for the treatment of high-grade astrocytomas. This treatment is accompanied by concomitant and post-radiotherapy treatment with temozolomide. Coadjuvant therapy with temozolomide and radiation therapy following surgical resection is required due to the infiltrative behavior of these neoplasms, which makes complete surgical resection virtually impossible.

Antitumor effects of EFAs in glioblastoma

Studies in cell cultures

Malignant gliomas are resistant to radio- and chemotherapy [23,24]. Expression of the DNA repairing enzyme *O*-6-methylguanine-DNA methyltransferase, which is active in approximately 50% of patients with GB, makes glioma cells resistant to alkylating agents [25]. On the other hand, radiation doses effective to kill glioma cells cause damage to adjacent normal tissue [26]. In order to increase the therapeutic efficacy of radiation in tumor cells a research group from Iowa, US, tested the effect of GLA, docosahexaenoic acid (DHA) and EPA in the radiosensitivity of 36 B10 rat astrocytoma cells [27]. They found that all three PUFAs, especially EPA, were cytotoxic per se to 36B10-malignant rat astrocytoma cells. When these PUFAs were added to cell cultures exposed to radiation, they increased the cytotoxic effect of radiotherapy [27]. Because GLA showed the strongest radiosensitizing effect, authors proposed that this PUFA might prove a useful adjuvant of radiotherapy in patients with GB. Importantly, although GLA induces oxidative stress and apoptosis in rat glioma cells, it does not affect the viability of normal rat astrocytes, an effect that was associated to a differential expression in antioxidant enzymes [28,29].

Previously, it was proposed that a relationship exists between PUFAs and primary brain tumors based in assay of peroxidative metabolism in 40 primary brain tumors [30]. It has been demonstrated that GLA, and to a lesser extent AA, increased the rate of peroxidation and apoptosis and inhibited cell growth. It has been hypothesized that in rapidly growing tumors, such as primary brain tumors, there may be a local deficiency in AA that leads to a loss of proliferative control [30]. These facts may be related to a limited generation of reactive oxygen intermediates (ROI) concluding that supplementation with exogenous AA and PUFAs may restore homeostasis [31,32]. To address this, primary brain tumor cell cultures from seven patients and non-tumor tissue were obtained and incubated with AA. Exogenous AA induced apoptosis preferentially in glioma cells, as assessed by the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling method [32]. The ability of exogenous AA and GLA to increase ROI production in human primary glioma cell cultures was also reported. Brain tumor tissue was obtained from 30 patients diagnosed with GB, and normal brain samples. Cells were exposed to GLA and a significant increased in ROI production was observed. The response was greater in higher-grade tumors. The benefit of this study was that using tumor fragments, a heterogeneous population of tumor cells simulating a model that better mimics the clinical scenario was found. The researchers obtained evidence that 4 to 40 μmol/L of AA or GLA stimulated apoptosis in human brain tumor cells [31]. Furthermore, the researchers tried to create a model that simulated three-dimensional brain tumors [33]. They generated glioma spheroids derived from several human and rat glioma cells and exposed them to GLA. A dose-dependent response to GLA was found: Although low levels of GLA optimize the growth of peripheral (infiltrating) cells, high doses of GLA led to an increased apoptotic index in both the periphery and the center of the glioma spheroid [33]. Thus, an effective dose of GLA to induce apoptosis in glioma spheroid is three times greater than needed to kill tumor cells in monolayer cultures [33]. These findings suggest that high local doses of GLA need to be achieved for toxicity against glioma cells. However, the low toxicity of GLA toward normal brain parenchyma may allow the local delivery of high doses of GLA without significant risk for neurotoxicity.

Other research confirmed that primary human glioma cells increased peroxidation and apoptosis when exposed to AA, suggesting that ROI formation may participate in tumor cytotoxicity [34].

Another report evidenced that after prolonged exposure to PUFAs, C6 rat glioma cells were protected against oxidative stress. This was linked to an increased activity of glucose-6-phosphate dehydrogenase, with consequent nicotinamide adenine dinucleotide phosphate reduction and increased levels of reduced glutathione, which protects against the toxic action of free radicals. PUFAs also decreased mitochondrial membrane potential, and consequently inhibit proliferation and cell migration. Additionally, there was no increase in prostaglandin synthesis. This latter finding may explain the absence of inflammation in C6 glioma models in rats exposed to GLA [35]. A decreased expression of the Ku80 protein, Ku70/Ku80 complex has been demonstrated. These proteins have an important role in DNA repair in tumor cells. Thus, this fact could explain the increased sensitivity in cells treated with PUFAs to radio- and chemotherapy [36]. One study also used C6 rat glioma cells exposed to Ru(2)GLA. It was synthesized with the goal of combining and possibly improving the antitumor properties of two active components, ruthenium and GLA. Decrease mitochondrial membrane potential and increased reactive oxygen species generation may be involved in the proapoptotic mechanism of Ru(2)GLA [37].

MicroRNAs are RNA molecules that could be regulators of gene expression and also may interfere with chemoresistancy, angiogenesis, invasion, metastasis, or immunogenicity. It has been proposed that apoptosis in glioma cells could be triggered by microRNAs due to PUFA treatment [38].

Studies in rodent models

The antitumor effect of PUFAs also was observed in vivo in preclinical glioma models. Local infusion of GLA in C6 glioma-bearing rats induced tumor cell death and reduced tumor cell proliferation, leading to tumor regression without affecting the normal brain parenchyma [39,40]. Intraparenchymal and intratumoral routes were proposed as effective methods to administer GLA in the treatment of gliomas [41]. However, in agreement with in vitro studies from the same group, the antitumor effect of EFAs seems to be dependent on the dose used. Although low concentrations of EFAs increased tumor growth in murine glioblastoma multiforme models, high concentrations inhibited cell proliferation [40,42]. Importantly, it was found that although apoptosis was readily detected in glioma tissue after 3 to 7 d of PUFA infusion using mini-pumps, adjacent brain tissue and vasculature were preserved [40]. This was the most complete study published so far because it included multiple glioma models with different features [40].

Intratumoral administration of GLA in glioblastoma-multiforme-bearing rats not only inhibits tumor cell proliferation and induces apoptosis, but also exhibits an antiangiogenic effect [43]. It has been proposed that GLA affects tumor cell cycle by modifying the intracellular levels of cyclin D1, retinoblastoma protein (pRb), p53, and p27. The effect of GLA on tumor angiogenesis appears to be mediated by changes in the expression of vascular endothelial growth factor, vascular endothelial growth factor receptor 1, extracellular-signal-regulated kinases 1 and 2, and matrix metalloproteinase-2 [43].

Studies performed in human and dogs

In 1991, the tumoricidal action of *cis*-unsaturated fatty acids and their relationship to free radicals and lipid peroxidation were reported. It was suggested that methods designed to specifically enhance superoxide radical generation and lipid peroxidation in the tumor cells may constitute a novel approach to cancer therapy [44].

~~A~~ further research was carried out in a small sample of patients (N = 6). The patients underwent surgical procedures for gliomas treatment and after GLA injection. Criteria for inclusion in the study included histologic and radiologic evidence of malignant glioma; clinical and/or radiologic evidence of residual tumor after the patient's last course of radiotherapy and/or surgery, and a tumor of sufficient size to warrant further therapy. Beginning 10 d after surgery, GLA injections were performed by a catheter located in tumor bed or by injection under sterile conditions. Most patients received GLA at the rate of 1 mg/d for 10 d consecutively. Some patients received glucocorticoid treatment as well as GLA injections. Significant necrosis was observed in computed tomography (CT) scans after GLA therapy. The results suggested that GLA may be beneficial in gliomas treatment without any side effects observed during therapy [45].

Other studies were carried out in three healthy dogs, used as control, and 15 patients with radiologic and histologic evidence of advanced gliomas. Eleven patients had primary tumors and 4 had recurrent lesions. All patients underwent a neurosurgical procedure with subtotal to near total resection of the tumor, and GLA was instilled intraoperative into the tumor bed and after through a cerebral catheter placed in the cavity. On postoperative day 7, a CT scan of the brain was carried out. One mg of GLA was instilled daily for 10 d. Suitable antibiotics and glucocorticoid therapy were given. A CT scan was performed after 10 d of treatment, and standard radiotherapy was performed afterward. None of the dogs developed side effects or complications. Study results support the idea that intratumoral instillation and injection of GLA may induce necrosis and cyst degeneration of the tumor, as evidenced by changes seen in CT scans. The fact that 12 of the 15 patients with grade III to IV malignant brain tumors showed no tumor recurrence over a period of 16 to 20 mo of follow-up, suggested that GLA is a useful approach in the treatment of human gliomas [5]. In a long-term study, researchers prolonged the follow-up of 14 patients over 20 to 26 mo. At the end of this period, 8 patients were alive and required no new surgery. This may indicate a beneficial effect of *cis*-PUFAs, without side effects or complications. It is important that normal cells producing superoxide dismutase manage, without harmful effect, these peroxidation processes induced by GLA administration. On the other hand, tumor cells, due to lack of oxidative pathways, cannot do it and thus are more susceptible to the cytotoxic effects of *cis*-PUFAs [46].

Nine adult patients who were diagnosed with recurrent high-grade gliomas after surgery, radiation, or chemotherapy, were evaluated following the effect of GLA (1 mg) for 7 d via a cerebral reservoir placed into the tumor bed or by direct intratumoral delivery. When pre- and post-GLA scans were compared, a considerable decrease in the contrast enhancement and peritumoral edema, and an increase in cystic areas were observed. The possible antiangiogenic action of GLA was postulated as responsible for the reduction in contrast enhancement [47].

Taken as a whole, these findings emphasize that the main tumoricidal action mechanism of GLA is its ability to enhance free-radical generation and lipid peroxidation process. Additionally, GLA-inactivated Bcl-2, promoted release of cytochrome C from mitochondria, and activated caspases inducing apoptosis. According to this, a relation with poly(ADP-ribose) polymerase-1 may be considered [48,49].

Other studies

The composition of fatty acids seems to differ between glioma and non-malignant brain samples [50]. A reduction in the content of DHA and an increase in the levels of LA were observed in tumor samples from patients with GB when compared with non-malignant brain from normal patients [50]. Dietary consumption of different fatty acids may affect their composition in glioma tissue. It has been recently shown that feeding C6 glioma-bearing rats with DHA oil increased EPA content and the ratio of ω-6/ω-3 fatty acids in the tumor is decreased [51].

Several other reports indicate that certain PUFAs may constitute a useful adjuvant therapy for the treatment of GB. GLA has been shown to sensitize rat glioma cells, but not normal astrocytes to the cytotoxic effect of radiation therapy [27]. PUFAs also seem to sensitize glioma cells to the cytotoxic effect of chemotherapeutic agents. The effect of PUFAs in doxorubicin toxic action was studied in 2010 in an in vitro model. It was observed that high concentrations of PUFAs are necessary to increase antitumor activity of this chemotherapeutic drug. The effect was mostly observed with DHA [52].

Conclusions

Several research groups have studied the effect of PUFAs and EFAs in gliomas. Each group was devoted to a particular aspect of this interaction but a correlation among them needs more analysis. We highlight the progress made by these groups.

One group studied the efficacy of GLA on human gliomas showing that PUFAs such as GLA could be of significant benefit in

the management of glioma. The study also outlined the importance of imaging techniques (such as CT scans and magnetic resonance imaging) in the evaluation of human glioma following treatment [44–49]. However, the possibility of final histopathologic analysis (autopsy) has greater advantages over that obtained by images. By histopathologic techniques, one could establish the status of molecular markers that can be compared between the pretreatment and post-treatment tissues. A U.K. study strived to create a model that mimics human GB [39–42]. Cells were extracted from human brain tumors and cultures performed to recreate their cellular heterogeneity [41]. Glioma spheroids in collagen gels also were used to achieve the three-dimensional features of brain tumors [33].

In a study in Brazil, the time required for exposure of tumor cells to GLA, EFAs effect on mitochondrial membrane stability, and advances in molecular area were studied [35–37,43].

Other studies have focused on the beneficial synergistic use of EFAs, which would promote methylation and inactivation of tumorigenic genes, with temozolomide and radiotherapy [23,25].

The international workshop "Brain Uptake and Utilization of Fatty Acids" concluded that this research areas needs more studies and their valuable recommendations were published in 2001 [53].

Great progress has been achieved in the last decade toward the use of EFAs for the treatment of patients with glioma, who show a dismal prognosis. The use of PUFAs as adjuvants in the treatment of gliomas is a promising therapeutic approach because they exhibit tumoricidal activity per se and enhance the antitumor effects of radio- and chemotherapy in the absence of adverse effects.

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