NATURAL AND SYNTHETIC DYE COMPOUNDS: APPLICATIONS IN GLIOBLASTOMAS THERAPY

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ABSTRACT

Glioblastomas are the most aggressive high-grade gliomas, characterized by diffuse infiltrative growth, high migration potential, angiogenesis, predisposition for necrosis that are decisive steps in resistance to therapy. In spite of current advances, the treatment of these tumors remains a challenge for oncologists. Because of the poor prognosis of these patients, development and testing of more effective therapeutic strategies is undertaken by several medical scientific communities. One of the new classes of drugs that are tested *in vivo* and *in vitro* is represented by the dye-compounds. This review focuses on efficacy of these drugs and their mechanisms of action in glioblastomas.

Keywords: dye compounds, glioblastoma, therapy

INTRODUCTION

The most studied primary brain tumors in adults are high-grade gliomas (HGG) which represent over 60% of all primary brain tumors. HGGs arise by malignant transformation of astrocytes. According to the 2007 WHO (World Health Organization) classification of central nervous system tumors, high-grade gliomas are: diffuse astrocytomas (WHO malignancy grade II), anaplastic astrocytoma s (WHO malignancy grade III), and glioblastomas (WHO malignancy grade IV). (1) Glioblastomas (GB) are the most aggressive HGG, representing the most common malignant tumor (45.6%) and the majority of gliomas. They may arise *de novo* or by malignant progression from astrocytoma. During the process of malignant transformation occurs a subsequent alteration in tumor-associated pathways, such as: PI3K/Akt, Ras/Raf/MAPK, p53/MDM2/p14ARF. (2) In spite of current advances, the GB treatment remains a challenge for oncologists. In ESMO (European Society for Medical Oncology) and NCI (National Cancer Institute) *guidelines* for the *treatment of GB,* it is recommended surgery as initial approach for newly diagnosed patients, followed by radiotherapy associated with concomitant or adjuvant termozolomide therapy. (3) Because of the poor prognosis of these patients, *development and testing of more effective* therapeutic strategies (including some new chemotherapeutic agents) is undertaken by several medical scientific comunities. Among the tested substances are dye-compounds. Dyes form a major class of natural and synthetic compounds known from antiquity for their properties as colorants but also known for a variety of therapeutic properties including anti-inflammatory, antioxidant and antiseptic activity. (4) These drugs are either natural (e.g. curcumin, quercetin, saffron, etc), or synthetic products (e.g. helianthin). In recent years, some dye compounds have shown promise both as potential antitumor agents

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alone or in combination with conventional treatment for several types of malignancies. The substances gained attention as chemotherapeutic agents because of their cytotoxic effect on malignant cells as well as their favorable toxicity profile *in vivo*. (5) Dyes have been demonstrated to have antiproliferative effects on cancer cells by inhibiting RTK's (receptor tyrosine kinases) signaling and inducing apoptosis in cancer cells. (5,6) Some of them are also able to inhibit intracellular signal transduction pathways *in vivo*. (5,6) These compounds are reported to be involved in mutagenesis and carcinogenesis. These properties of dyes open a therapeutic window for the treatment of malignant diseases, including glioblastomas.

The current review describes the recent findings regarding the biological activity of some natural (curcumin, quercetin) and synthetic (helianthin, methyl red and methyl yellow) dyes. The review presents the efficacy of these drugs and their mechanisms of action in several types of cancers focusing on glioblastomas.

NATURAL DYE COMPOUNDS

Curcumin

Curcumin (diferuloymethane) is a polyphenol derived from the rhizome of the herb *Curcuma longa*, popularly known as turmeric. The substance is hydrophobic therefore is poorly soluble in water or other neutral solvents, therefore it's bioavailability is poor. The substance is rapidly metabolized in intestine and also in the liver, so in the end approximately 60-70% of an oral dose gets eliminated in the feces. (7) In clinical studies, curcumin was administrated orally in high doses but the plasmatic levels were also very low. In the last 2 decades the bioavailability of cucumin was enhanced by using new delivery systems like: micelles, (8) liposomal vesicles (9), nanoparticles (9,10), nanoemulsions (11), and phospholipid complexes. (12) The rhizome of Curcuma longa contains a complex of curcuminoids which is formed by: diferuloymethane, demetoxycurcumin, bisdemetoxycurcumin and cyclocurcumin. Commercial curcumin contains only the first three curcuminoids. (13) Turmeric has been used since ancient times (over 2000 years ago) in Asia as a spice, for fabric dyes, cosmetics but also in Indian Ayurvedic medicine for treatment of some disorders like: infections, burns, allergies, rheumatisms, liver disorders, and so on. (14) Nowadays, curcumin is used in *in vivo* and *in vitro* studies, for treatment of several diseases such as antiseptic, antioxidant, anti-inflammatory, but also anti-proliferative, anti-metastatic anti-angiogenic and antimutagenic. (15,16) The substance was used for cancer treatment (17,18) but also for prevention of various types of cancer. (19,20) After the positive results of the first clinical trial in 1987, when turmeric was used for skin cancer treatment, (21) curcumin (either alone or in combination with other chemotherapic agents) was used in numerous clinical trials for the prevention or treatment of various cancers like: colorectal cancer (19), pancreatic cancer (22), breast cancer (17), prostate cancer (23), multiple myeloma (24), lung cancer (25), oral cancer (26), head and neck cancer. (27) In the last decades, several research groups investigated the efficacy of curcumin treatment in GB cell lines. The first report which confirmed the antiproliferative effect of turmeric in GB cell lines dates from 2003. After that, researchers observed that curcumin treatment induced the activation of pro-apoptotic signals and inhibited anti-apoptotic genes. (29) (Fig. 1) The substance is also capable to inhibit NF-kB signaling pathways, to decrease the expression of genes like Bcl family or IAP (which normally confer resistance to chemotherapy), to inhibit the expression of MGMT, Ku70, Ku 80 and DNA-PKcs (DNA repair enzymes), which are believed to be involved in GB cell resistance to chemotherapy and radiotherapy. In a study by K.M. Dhandapani et al, turmeric was shown to up-regulate the p53 expression in GB cells. (30) After the inhibition of some anti-apoptotic pathways, like Bcl2, NF-Kb or IAPs, it was found that curcumin can activate PARP and caspase 3 cascades and in consequences to initiate GB cells apoptosis. (29,31) Curcumin is also able to inhibit ING4 signaling pathway in GB cell lines (32) and also activates a non-apoptotic autophagy signal in GB cells, as a result of Akt/mTOR/p7056K pathway inhibition and ERK pathway activation. (33) (Fig.1) Curcumin induces differentiation cascade in GB – initiating cells, both *in vitro* and *in vivo*, followed by decreased self-renewal and clonogenic ability. (34) Furthermore, curcumin treatment inhibits the expression of MMPs (-1,-3, and -14) molecules through the suppression of PKC and MAPK signaling pathways (35) and by the inhibition of AP-1. (36) It is also capable to inhibit G6PT gene expression (37) and to activated proteolytic pathways through both mitochondria or death-receptor (38) or to induce apoptosis mediated through TRAIL/Apo2L. (39) It was also observed that the substance can sensitize GB cells to other chemotherapeutic agents or to radiotherapy. (30) There are only a few

in vivo studies that used curcumin as a treatment in GB tumors. The first *in vivo* study which results were presented in 2007 used subcutaneous xenograft model of GB. The administration of curcumin inhibited tumor growth and induced autophagy. (40) The next study published in 2010 demonstrated that the administration of turmeric to immune compromised mice diminished the growth of the tumor and improved survival. The compound was able to easy penetrate the blood brain barrier, altering the activities of matrix metalloproteinase -9, down-regulating CD31 and CD105 mRNA, and reducing the hemoglobin content in GB cells. (41) In a study by Zanotto-Filho, A, published in 2012, curcumin was used to treat GB immune-competent rats. The drug was administrated intraperitoneal and determined a decrease of 80% of the tumor volume. The substance proved no toxicity and the normal tissues were minimally affected by the treatment. The authors observed that the treatment affected some intracellular pathways like: PI3-Akt, NFkB, inhibited Bcl-2 expression and altered the mitochondrial activity independently of p53 and PTEN. (42) The preliminary results of a clinical trial which used curcumin in association with other treatments for a personalized and targeted therapy in pediatric brain tumors were also published in 2012. (43) The results are promising showing that individualized targeted therapy might be superior to traditional therapy.

Quercetin

Another natural dye compound used in GB therapy is quercetin (3,3',4',5,7-pentahydroxyflavone). The substance is a flavonol extracted and isolated from *Sophora Japonica L*. The substance is found in vegetables, fruits, tea, olive oil, and so on. Due to its chemical structure quercetin easily forms co-

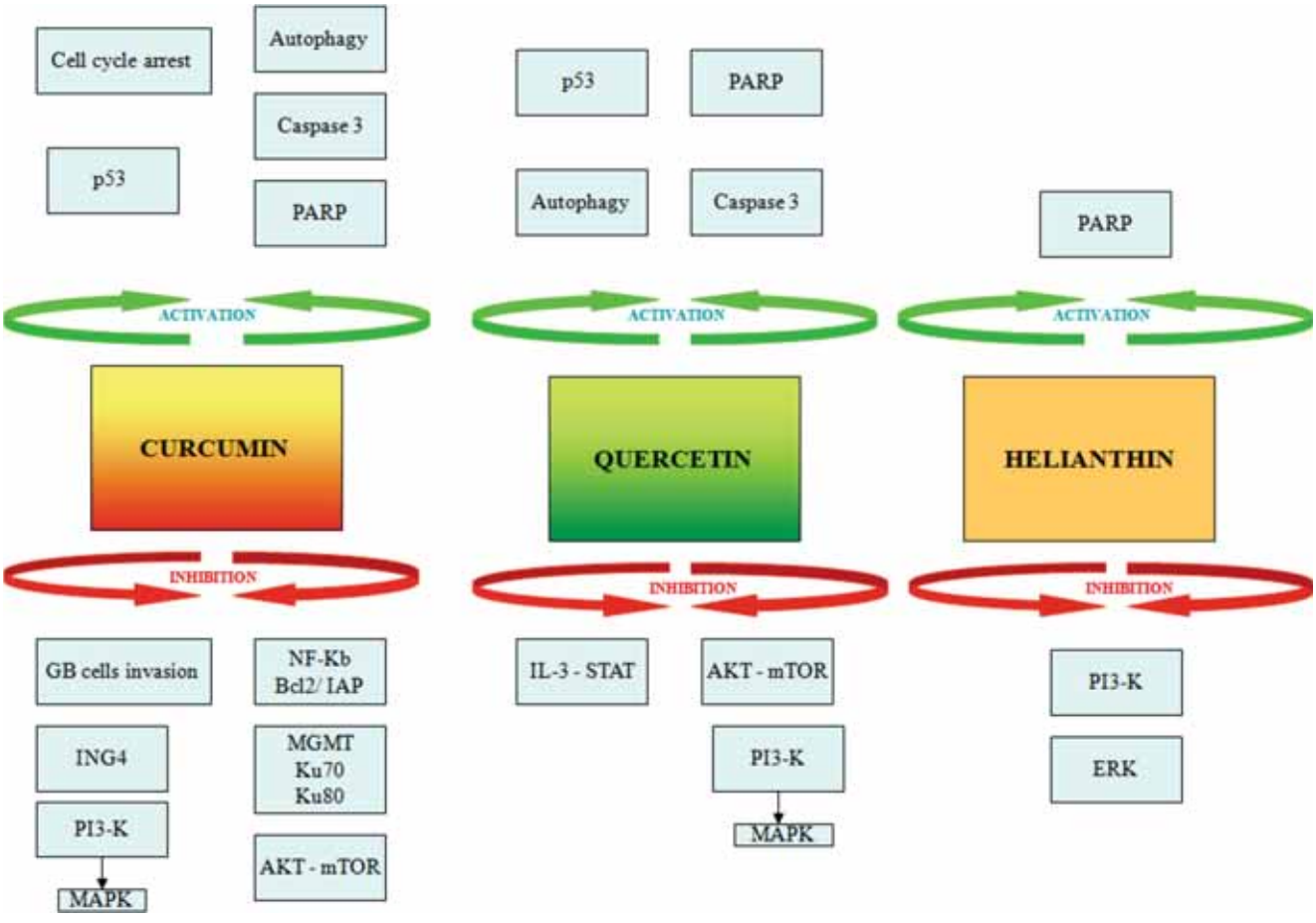

FIGURE 1. *Molecular targets of curcumin, quercetin and helianthin in glioblastomas*

Abbreviations

GB – Glioblastoma; **PARP** – Poly (ADP-ribose) polymerase; **P53** – Protein mass of 53 kDa; **ING4** – Inhibitor of growth protein 4; **PI3-K** – Phosphoinositide 3-kinase; **MAPK** – Mitogen-activated protein kinase; **NF-Kb** – Nuclear factor kappa-light-chain-enhancer of activated B cells; **Bcl-2** – B-cell lymphoma 2; **IAP** – Inhibitors of apoptosis proteins; **MGMT** – O6-methylguanine-DNA methyltransferase; **Ku70** – 70-kDa protein; **Ku80** – 80-kDa protein; **AKT (PKB)** – protein kinase B; **mTOR** – Mammalian target of rapamycin; **IL-3** – Protein kinase B; **STAT** – Signal Transducer and Activator of Transcription

loured complexes with metal ions. The drug is poorly soluble in water. The bioavailability is also reduced due to its poor absorption and rapid metabolism. In 2000, it was reported that quercetin glucosides are completely hydrolyzed in ileostomy patients before absorption. (45) In the last years, researchers focused on developing new drug delivery approaches for quercetin, like: prodrugs (46,47), inclusion complexes (48), nanocrystals, microemulsions (49), liposomes, phospholid formulations (50), quercetin-encapsulated polymer nanoparticles (48), quercetin polymeric micelles (51) etc.

Quercetin has many molecular mechanisms of action. It is capable to influence the activity of several enzyme systems both *in vitro* and *in vivo*. Among these enzymes are protein kinases. In fact, quercetin was probably the first described tyrosine kinase inhibitor. (52) and it was the first tyrosine kinase inhibitor tested in phase I clinical trial.(53) The substance is also capable to down regulate the mutant p53 protein, facilitating cell death. (54) and can arrest cancer cells (leukemia, gastric) in the late G1 phase of the cell cycle. (55)The drug has estrogen receptor binding capacity (56), can inhibit heat shock proteins (57) and the expression of Ras (58) and phosphatidyl-3 kinase proteins. (59) and I-phosphatidylinosotol-4 kinase (60) involved in signaling pathways. Quercetin treatment inhibited malignant cell growth in cancers like: breast, ovarian (61,62), gastric (63), colon, bladder, head and neck, lung (65), leukemia (66), melanoma. (67) (The animal studies that used quercetin demonstrated that the substance seems to be a selective inhibitor of the growth of malignant cells. (68,69) The FDA (Federal Drug *Administration)* has approved the administration of quercetin in humans in 1975. The dose of 100mg quercetin was well tolerated and did not induced side effects. (70) The results of the first clinical trial that used quercetin as treatment for patients diagnosed with cancer no longer responsive to standard therapies were published in 1996. (53)

In the last decades, several research groups investigated the efficacy of quercetin treatment in various GB cell lines. The first report, which confirmed the antiproliferative effect of , 3',4',5,7-pentahydroxyflavone in GB cell lines, dates from 1996 (71) and it was followed by a number of studies that demonstrated that quercetin exerts its by its antiproliferative effect on GB cells by different mechanisms: inhibition of PI3K-AKT/PKB pathway and activation of ERK-dependent COX-2/PGE (2) (73) and abrogation of IL-3/STAT3 signaling. (74) It was also proved that quercetin promotes apoptosis mediated by caspase-3and influences the feedback balance of MDM – P53 in GB cells. (75) (Fig.1)

The drug is capable to inhibit Hsp and to sensitize GB cells to termozolomide treatment. (76,77) Used in combination with sorafenib, quercetin induces programmed cell death in GB cells. The same effect of the substance was found when used in combination with imperatorin. (77) Until now, there are no clinical studies using quercetin as a treatment on GB patients.

Synthetic azo-dyes

Until now only helianthin, methyl red and methyl yellow effects were studied in GB therapy in vitro. In our previously studies, we found that among these 3 substances only helianthin had the capability to induce cytotoxicity in GB cell lines. Helianthin induces a reduction of EGFR, IGF-1R, PI3-K and ERK1/2 phosphorylation in GB cells. The drug also induced cleavage of PARP, without affecting the expression of Bcl2 in the studied GB cell lines. Until now there are no *in vivo* studies that used helianthin as an antitumor agent. (78) (Fig.1)

CONCLUSIONS

Clinical results from conventional therapy have been limited. New class of drugs, potentially useful for the treatment of GB patients is required. Dye compounds could be a very promising drugs in cancer treatments. Although much more work is required to test their effect in vivo and in vitro, it is hoped that these compound will help improve the efficacy of anticancer regimes in glioblastoma

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