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Review paper

Tamoxifen as a potential treatment of glioma

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Cerebral gliomas have a poor survival time even after multimodal treatment, because of the unavoidable recurrence of tumor. Several trials with a combination of old and new chemotherapics have been performed, but survival time remains generally less than 12 months. Tamoxifen (TAM) has recently been shown to inhibit the growth rate of established and low-passage human glioma cell lines. Furthermore, this drug has enabled stabilization of the clinical and radiographic picture in selected patients with recurrent glioma. Here we review published data to discuss a potential role of TAM in the multimodal postoperative treatment of cerebral gliomas. [© 1998 Lippincott Williams & Wilkins.]

Key words: Chemotherapy, high-grade gliomas, protein kinase C, tamoxifen.

Introduction

Even after macroscopically total excision of tumor and multimodal oncological treatment, malignant gliomas unavoidably recur after a variable period of time. Without any adjunctive therapy, the mean postoperative survival of patients is 4–5 months; it is 7–8 months after adding a cycle of radiotherapy (45–60 Gy) and 9–10 months after chemotherapy.[1–6] Combinations of different chemotherapies produced slightly improved results, but with a survival rate that seldomly exceeded 12 months.[3–8,10–15]

More recently, inhibitors of the enzyme protein kinase C (PKC) have been included in these therapies. In particular, tamoxifen (TAM) is of interest in this regard because it can be given orally and has low toxicity.[16–21]

Mechanisms of action of TAM

TAM is generally used in the treatment of estrogen receptor (ER)-positive breast cancer.[22,23] Beyond this well known anti-estrogen action, TAM also has been shown to exhibit cytotoxic activity against ER-negative cells. These effects are presumably related to cellular and extracellular mechanisms which involve the local enviroment, with activation or inhibition of molecular systems different than by ER, that might explain the efficacy of TAM in 10–15% of ER-negative breast cancers.[24]

Within a wide range of concentrations, TAM interacts with several intracellular systems, including enzymes fundamental to the regulation of cellular growth. The drug and some active metabolites are active at a plasma concentration of 1–2 μM,[25] allowing several effects observed *in vitro*. In some experiments *in vitro*, the effects have been obtained with nanomolar concentrations and are not reproducible *in vivo*. However, because of the high lipophilicity of the molecule, the intracellular concentration of TAM and of its metabolites could be higher than that detected in serum.

The estrogen-independent activities of TAM at micromolar doses are: (i) reduction of fluidity of the cell membrane and stabilization of its structure, with an antimetastatic effect (this effect can be obtained because of the lipid anti-peroxidative property of TAM), (ii) inhibition of PKC, with an antiproliferative effect, and (iii) inhibition of calmodulin and of Ca^{2+}-calmodulin-dependent phosphodiesterase, with a possible antiproliferative effect.[26–33]

The activities of TAM obtained with intermediate dosages are: (i) inhibition of the multidrug resistance protein (MDR), (ii) interaction with oncogene *erb*-B2, and (iii) inhibition of Estrogen Binding Sites type II receptors.[34–37]

At nanomolar concentrations TAM provokes: (i) inhibition of the so called Antiestrogen Binding Sites (AEBS), (ii) reduction of circulating prolactin (PRL) and interaction with the PRL receptor, (iii) interaction with adenyl cyclase, and (iv) interaction with tyrosine kinase.[25]

Other effects of TAM not related to ER are: (i) inhibition of the insulin-like growth factor (IGF)-1, an

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epithelial growth factor, (ii) increase of transforming growth factor (TGF)-β, which has been shown to inhibit proliferation of breast cells and to have anti-angiogenetic action, (iii) direct inhibition of angiogenesis, (iv) block of calcium channels, with possible antiproliferative effect, and (v) interaction with the cholinergic and histaminergic receptors, with consequent block of release of growth factors.[38-43]

Experimental studies demonstrated that TAM inhibits P-glycoprotein,[35,44] which may prevent the efflux of chemotherapy drugs from tumor cells, with enhancement of their antineoplastic activity. In particular, TAM sensitizes human malignancies to derivatives of platinum.[45] Modulation of cisplatin cytotoxicity by TAM has been demonstrated in melanoma, ovarian carcinoma and small cell lung cancer human cell lines.[45-47] In all these tumors a cytotoxic synergy between the two drugs has been observed at concentrations that can be achieved therapeutically.

TAM attains its therapeutic level in human serum 4-6 weeks after the onset of oral therapy;[20,23] therefore, this drug does not seem suitable for treatment of patients with a survival expectation lower than 2 months. Beyond the conventional dose of 40 mg/day, TAM also is well tolerated at doses of 150-200 mg/day.[16,17,48,49] At the dose of 40 mg/day the maximum plasmatic level is 2000 ng/ml; with higher dosages the plasmatic concentration of drug is 3.2 μM/l and that of its main active metabolite, *N*-desmyl-TAM, is 4.3 μM/l;[20,23,24,50] *N*-desmyl-TAM has an efficacy about 10 times higher than that of TAM.[23] Ducharme *et al.*[51] have evaluated TAM metabolic profiles in 25 patients suffering from recurrent malignant cerebral gliomas who were treated with high oral doses of TAM (120 mg/m^2 twice daily) for at least 8 weeks. Steady-state plasma concentrations of 2.94 ± 3.44 μM/l were observed for TAM, 4.37 ± 2.13 μM/l for *N*-desmethyl-TAM, 1.49 ± 0.54 μM/l for *N*-desdimethyl-TAM and 0.13 ± 0.05 μM/l for 4-hydroxy-TAM. Mean plasma TAM concentrations were higher in dexamethasone-treated patients than in untreated patients: 3.94 ± 4.35 versus 1.67 ± 0.84 μM/l, while phenytoin-treated patients had lower concentrations: 1.85 ± 0.87 versus 4.58 ± 5.05 μM/l. The cerebrospinal fluid concentration of TAM is about 1-2% of the plasma level, because of its difficulty in crossing the blood-brain barrier,[50,52] which, however, is partially disrupted after neurosurgical procedures.

The possible use of TAM in the multimodal treatment of malignant gliomas is related to the following effects: (i) inhibition of DNA synthesis by TAM in human glioma cell lines, (ii) inhibition by TAM of cellular proliferation induced by epidermal growth factor (EGF), observed in low-passages human glioblastoma cell lines, (iii) enhancement by TAM of the antitumoral activity of alkylating agents and radiotherapy, in mouse glioma cell lines, (iv) fragmentation of DNA of human glioma cell lines, and (v) inhibition of the PKC of human glioma cell lines.[27,28,30,53-56]

The property of TAM to inhibit potently the PKC at micromolar concentrations[17,28,30,54,55,57] may be fundamental for its use against gliomas. In fact, unlike normal glia, human glioma cell lines show a high PKC activity,[30] closely related to their growth rate observed *in vitro*.[27,28,30,54,55,58,59] PKC is a phospholipid- and calcium-dependent kinase, belonging to the serine/treonine kinases family, with various isoforms encoded by different genes.[59] This enzyme acts as the receptor of tumor-promoting agents (TPA), which are phorbol esters that promote neoplastic transformation and tumoral growth.[59] PKC appears to be involved in transduction of mitogenic signals from the cell membrane to the nucleus; it is co-activated by diacylglycerol and by calcium, which are disposed in the intracellular space after activation of phosphatidylinositol metabolism.[59] By phosphorylating several cellular substrates, PKC provokes alteration of the cytoskeleton, desensitization of membrane receptors, modulation of ion channels and regulation of genic expression (activation/disactivation of specific transcription factors).

The inhibition of PCK by TAM is probably dependent on a specific interaction with phospholipids which activates the enzyme. The TAM-PKC interaction occurs on the catalytic site of the enzyme, at concentrations ranging from 10 to 100 μM.[30] *In vivo*, PKC inhibition has been observed at concentrations of TAM higher than 5×10^{-6} M, compatible with the cytotoxic effects of drug, but not reconcilable with the antiproliferative action obtained with lower dosages in several cell lines.[30]

Its ability to inhibit PKC and, probably, other transduction mechanisms enables TAM to cause a dose-dependent reduction of DNA synthesis in human glioma cell lines at concentrations that can also be achieved therapeutically.[27,28,30,32,55,57,58]

Clinical results obtained with TAM against gliomas

In view of encouraging results with glioma cell lines[27,28,54-56] and to the safety of drug, proved in large series of breast cancer patients,[22,23] during recent years some authors have proposed that oral administration of TAM be used in failed patients with recurrent malignant gliomas (Table 1).[16-18,20,21,27,60,61]

Vertosick *et al.*[20,21] originally observed that seven patients treated with 40 mg/day of TAM survived almost 6 months with an unchanged KPS and CT picture, and that two cases had CT evidence of tumor regression, without side effects. After 1 year treatment with 180 mg b.i.d. TAM administration, Baltuch *et al.*[16] achieved clinical stabilization and CT tumor regression in a patient with a recurrent glioma, previously treated with surgery and radiotherapy. In a series of patients with recurrent malignant gliomas, Couldwell *et al.*[17] observed neurological improvement and radiological 'response' (decrease of tumoral volume greater than 50% on MRI) in three of 11 patients treated with high doses of TAM (160–200 mg/day), while in another case the clinical and CT picture was unchanged after 6 months. In another study of the same authors on 32 cases (20 glioblastoma and 12 anaplastic astrocytomas), after a long-term follow-up the proportion of cases with stabilization/regression of tumor was similar: eight patients showed a response and six had a stabilization of disease.[62]

In 13 failed patients treated with 40 mg/day of TAM, Gewirtz *et al.*[18] obtained a median survival of 38 and 27 weeks (patients re-operated and not, respectively), versus 27 and 22 of the control group. However, these differences were not statistically significant (p=0.17). In a series of 30 patients with recurrent high-grade gliomas treated with 120 mg/m^2 b.i.d. of TAM, Shenouda *et al.*[61] obtained about 50% reduction of tumoral volume in five cases and stabilization of disease in other cases, without significant side effects. Davis and Shaw[48] tested the safety of mega-doses of TAM (960 mg/day) in 14 patients with recurrent glioma: in eight cases TAM was administered in combination therapy with PCV, in two with cisplatin, in two with PCV and cisplatin, and in the other two alone. Despite the very high doses of TAM, they observed only minimal side effects; however, the study had a short course and did not consider any survival data.[48] Freeman *et al.*[63] administered TAM at unspecified doses to five children affected by intrinsic brain stem gliomas: during a follow-up period ranging from 2 to 26 months, in four cases a clinical and MR tumor regression greater than 50% was obtained, whereas in the fifth case the clinical course was unchanged.

Zhang *et al.*[49] reported a parallel *in vivo–in vitro* study in a patient with a malignant glioma, unresponsive to radiotherapy but responsive 22 months to 160 mg/day of TAM. Following tumor recurrence, the primary cell culture obtained from the second surgery showed a loss of sensitivity to TAM and a growth inhibition by hypericin, another potent inhibitor of PKC that causes apoptosis of glioma cell lines.[49] In a series of 12 patients operated on for a glioblastoma and treated with 200 mg/day of TAM and three cycles of carboplatin before radiotherapy, Puchner *et al.*[19] reported the following side effects: deep venous thrombosis, amenorrhea, ovarial cysts, mild leucopenia and thrombopenia, mild nausea, and slight deterioration of hearing. After a mean follow-up of 13 months, nine patients were still alive; four cases treated with partial surgical resection did not show progression of tumor and two of them had a tumor regression.

Chang *et al.*[64] reported a phase II trial on the efficacy and toxicity of the continuous administration

Table 1. Summary of the literature reporting glioma patients treated with TAM, alone or in combinational therapy with other chemotherapies

References	Number of cases	Histology	Dose of TAM (mg/day)	Response[a]	Partial response[b]
20	32	29 GBM, 3 AA	40	9	2
16	1	GBM	180	1	–
17	11	6 GBM, 5 AA	160–200	3	1
62	32	20 GBM, 12 AA	160–200	8	6
18	13	12 GBM, 1 AA	40 or more	NS	NS
61	30	NS	120	5	NS
63	5 children ND	brain stem gliomas	NS	4	1
48	14	10 GBM, 4 AA	960	NS	NS
49	1 ND	GBM	160	1	–
19	12 ND	12 GBM	200	2	4
64	18	8 GBM, 10 AA	240	–	4
65	40 ND	32 GBM, 8 AA	40–80–120	20	12

[a]Number of cases with reduction of tumoral volume > 50% and/or survival longer than 6 months (patients with recurrent gliomas) or 12 months (newly diagnosed).
[b]Number of cases unchanged after 6 months and/or survival between 6 and 12 months
GBM, glioblastoma; AA, anaplastic astrocytoma (including oligo-astrocytoma); NS, not specified; ND newly diagnosed.

of high-dose TAM (240 mg/m^2/day orally) and interferon-α (6×10^6 U subcutaneously three times per week) in 18 failed glioma patients (glioblastoma in eight patients, anaplastic astrocytoma in five, astrocytoma in four and mixed malignant glioma in one). The combination of TAM and interferon-α was associated with significant neurotoxicity (reversible side effects were dizziness and unsteady gait), resulting in early study closure. In 75% of cases a progression of disease was observed after one cycle of treatment and 25% had stable disease or minor response.[64]

Recently we published the results of our clinical study on the combining of TAM and i.v. carboplatin on 40 newly diagnosed patients with high-grade cerebral gliomas.[65] The oral dose of TAM ranged from 40 to 120 mg/day. The survival data have been compared with those of a control group of 40 patients treated with i.v. carboplatin alone. Two patients of the TAM group died in the postoperative period from pulmonary embolism and from myocardial infarction, respectively. The patients of the TAM group had a median survival rate of 13 months (control group: 9 months; p=0.04). The 12 and 24 month survival rates were 52 and 32% (control group: 30 and 0%), respectively. The median relapse-free survival time was 9 months in the TAM group and 4 months in the control group (p=0.0014). Patients treated with 80–120 mg/day of TAM seemed to have longer median survival rates (13 months both) and a better 12 month result (58 and 76%, respectively).

Conclusion

Even though the preliminary results seem to be encouraging, the small number of patients treated thus far does not allow a definitive judgement on the efficacy of TAM in the multimodal treatment of cerebral malignant gliomas. However, the possibility of oral administration, the tolerability and the slight side effects justify the use of TAM, expecially in combination therapy. On the basis of the available literature and our limited experience the reccommended dose of TAM seems to be 120–160 mg/day.

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