Case study

Temozolomide therapy in a man with an aggressive prolactin-secreting pituitary neoplasm: morphological findings

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Summary

Administration of temozolomide to a 46-year-old man with an invasive aggressive prolactin (PRL)–secreting pituitary neoplasm resulted in improvement of the clinical condition and significant decrease of blood PRL levels. Histologic, immunohistochemical, and electron microscopic study demonstrated marked morphological differences in the tumor exposed to temozolomide compared with the unexposed tumor. Necrosis, hemorrhagic areas, accumulation of connective tissue, focal inflammatory infiltration, and neuronal transformation were seen. Immunohistochemical prognostic indicators showed a reduction in growth potential. Based on the clinical, laboratory, and morphological findings, we recommend temozolomide therapy in patients with pituitary tumors not responding adequately to other treatment options.

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1. Introduction

Patients with pituitary tumors can be treated by surgery, various forms of radiotherapy, and different drugs. Dopamine agonists are effective in many patients with prolactin (PRL)–producing pituitary tumors [1]. They decrease blood PRL levels and cause tumor shrinkage and clinical improvement. Long-acting somatostatin analogs are used in the treatment of patients with growth hormone (GH) and/or thyrotropin (TSH)–secreting pituitary tumors [2]. Administration of these drugs diminishes blood GH and TSH concentrations, reduces tumor size, and ameliorates the clinical conditions. Pegvisomant, a GH receptor blocker, was introduced for treating patients with acromegaly [3]. Temozolomide was recently recommended as a new approach in the therapy of aggressive pituitary tumors [4,5]. Syro et al [6] administered temozolomide to a 46-year-old man with an aggressive PRL-secreting pituitary neoplasm. The clinical and laboratory findings were briefly reported in a letter. We had the opportunity to investigate and compare the
2. Clinical and laboratory findings

The patient was a 46-year-old man with a large, invasive, aggressive PRL-secreting pituitary neoplasm. In a period of 15 years, 5 surgeries were performed, but the tumor could not be completely removed. Radiation therapy and administration of dopamine agonists (bromocriptine and cabergoline) were ineffective. Blood PRL levels were markedly elevated (1885 ng/mL). After surgeries, blood PRL levels temporarily decreased but started to rise again, and the tumor increased in size. Blood follicle stimulating hormone (FSH), luteinizing hormone (LH), and cortisol levels were low. Blood GH, TSH, and thyroxine (T₄) concentrations were within the normal range. Hormone replacement therapy was given. The patient complained of visual disturbances, which were only temporarily improved after surgeries. Magnetic resonance imaging (MRI) disclosed a large tumor with chiasmal compression and suprasellar extension measuring 47 × 44 × 37 mm, and invasion to the cavernous sinus (Fig 1A). The fifth surgery was not successful. Only a small portion of the tumor could be resected because of hard consistency. MRI demonstrated a large neoplasm (50 × 45 × 50 mm), and the postoperative blood PRL level was 1838 ng/mL.

Temozolomide therapy was started in January 2005 at a dose of 200 mg/m²/d × 5 days. Treatment was repeated every 28 days for 7 months. The clinical condition improved, blood PRL levels decreased to 30 ng/mL, and MRI demonstrated hemorrhage, necrosis, and shrinkage of the tumor (Fig. 1B). In October 2005, another surgery was performed. The tumor was easily resected because of its friable and soft consistency and was submitted for histologic, immunohistochemical, and electron microscopic investigation. The morphological changes found in the tumor removed after temozolomide treatment were compared with those observed in the tumor from the last pre-treatment surgery.

3. Morphological findings

Details of the histologic, immunohistochemical, and electron microscopic methods were described previously [7]. The antibodies used for immunohistochemistry in this study were for PRL (monoclonal; Immunotech, Marseilles, France); GH and corticotropin (polyclonal) as well as β-TSH and β-FSH (monoclonal; all 3 from Dakocytomation, Carpinteria, CA); β-LH (polyclonal; NIDDK-NIH, Torrence, CA); and α subunit (monoclonal; Biogenex, San Ramon, CA). For Ki-67, the MIB-1 antibody was used (Ventana Medical, Tucson, AZ).

The tumor removed before temozolomide treatment was a cellular well-vascularized neoplasm exhibiting a diffuse pattern interspersed with narrow strands of connective tissue. It consisted of chromophobic and slightly acidophilic periodic acid–Schiff–negative cells. In some areas, marked congestion was evident. Cellular and nuclear pleomorphism was moderate and mitotic figures were easily identified (11/1000) nuclei (Fig. 2A). The streptavidin-biotin-peroxidase complex method demonstrated cytoplasmic immunopositivity for PRL in many adenoma cells. Immunostainings were negative for GH, corticotropin, TSH, FSH, LH, and α subunit of the glycoprotein hormones.

The morphological features of the tumor removed after temozolomide treatment markedly differed from those reported before temozolomide administration. By histology, the chromophobic slightly acidophilic periodic acid–Schiff–negative tumor cells were separated by edema and hemorrhage. In several areas, accumulation of connective tissue was noted. The tumor cells were irregular, showing

Fig. 1 A and B, Demonstrates the coronal magnetic resonance image of the sellar tumor before (A) and after (B) temozolomide treatment.
moderate cellular and nuclear pleomorphism. Mitotic figures were also encountered (2/1000 nuclei). In few areas, mild to moderate mononuclear cell infiltration was noted. Several tumor cells were very large and resembled nerve cells (Fig 2B). By immunohistochemistry, many tumor cells were immunopositive for PRL. Few tumor cells appeared to express GH as well. Immunostainings were negative for corticotropin, TSH, FSH, LH, and α subunit of the glycoprotein hormones. Several mononuclear inflammatory cells were immunopositive for CD3, a T-cell marker, and for CD68, a macrophage marker. Few inflammatory cells were immunopositive for CD20, a B-cell marker. Immunostaining for CD34, an endothelial cell marker, showed that the tumor was well vascularized. Several capillaries were abnormal in shape and size. E-cadherin expression in the cytoplasm of tumor cells was slightly decreased. Expression of neurofilament antigen, S-100 protein, and glial fibrillary acidic protein was apparent in several cells.

Comparison of immunohistochemical prognostic indicators showed marked differences between the pretreated and temozolomide-treated tumor specimen. In the pretreated tumor, the Ki-67 nuclear labeling index using the MIB-1 antibody was 40% to 60% (Fig. 2C); topoisomerase-2-α, 90%; P-27, 25%; P-53, 15%; vascular endothelial growth factor, 100%. In the tumor cells after temozolomide therapy, the Ki-67 index markedly decreased to 5% (Fig. 2D); topoisomerase-2-α, 80%; P-27, 70%; P53, 4%; and vascular endothelial growth factor, 95%.

By electron microscopy (Fig. 3A), the tumor (before temozolomide treatment) was highly atypical and undifferentiated. The characteristic features of PRL-producing cells were not apparent. The cells were spherical or irregular, closely apposed without well-formed intercellular junctions. The nuclei were ovoid, irregular, and occasionally bizarre, containing 1 or more variably developed nucleoli and small to moderate quantities of stippled or clumped heterochromatin. The prominence of rough-surfaced endoplasmic reticulum (RER) was variable, and well-organized arrays of RER were only seen in occasional tumor cells. In a few tumor cells, annulate lamellae were noted; this special RER
formation occurs in atypical cells. The Golgi complexes were rarely prominent. The secretory granules were small, extremely scant, and spherical, and it did not exceed 200 nm in most of the tumor cells. Granule exocytoses were only rarely detectable. Numerous tumor cells displayed variable degrees of oncocytic change. The ultrastructural features of the mitochondria were within the normal range.

Electron microscopy (Fig. 3B) documented a surprisingly heterogeneous tumor tissue (after temozolomide treatment), the components of which were (1) small cells displaying poorly developed membranous organelles and a few minute (50-150 nm) secretory granules. These cells resembled null cells, (2) middle-sized cells having proportionally larger nucleus and a prominent nucleolus. The well-developed cytoplasm contained appreciable quantities of RER and a sizeable Golgi complex. However, the 50- to 150-nm secretory granules were very sparse, and these cells did not possess markers of PRL cell differentiation either; (3) admixed with the tumor cells described hereinabove, there was a neural component consisting of neuropil (masses of what appeared to be neuronal processes including varying amounts of cytoplasmic constituents) as well as large neuronlike cells. The latter had very large, often pleomorphic, nucleus with markedly large nucleolus. The ample cytoplasm harbored peripheral parallel stacks of RER, heavily studded with ribosomes—the ultrastructural equivalent of Nissl substance. The Golgi apparatus seemed to be prominent, but the secretory granules were very small (50-150 nm) and scant. These cells, resembling the perikarya of secretory neurons, contained also aggregates of low-density microfilaments.

4. Discussion

Temozolomide is an imidazotetrazine derivative, an alkylating compound that depletes MGMT (0-6-methylguanine-DNA methyltransferase), and a DNA repair enzyme, which methylates DNA and exerts an antineoplastic effect against various experimental tumors [8]. It absorbs rapidly after oral administration and crosses readily the blood-brain barrier. Because of easy penetration to the central nervous system, patients with various gliomas and cerebral metastases of malignant melanoma were treated with the drug, and the results showed reduction of the tumor mass, clinical improvement, and prolonged survival [9-11]. A group of patients with pheochromocytoma, pancreatic endocrine neoplasms, and carcinoid tumors showed objective biochemical and radiologic improvement followed by oral treatment with temozolomide and thalidomide, suggesting that the combination of these 2 drugs appeared to be an option in the treatment of neuroendocrine tumors [12]. Fadul et al [4] administered temozolomide to 2 patients with pituitary carcinoma. The first patient had a PRL-secreting pituitary carcinoma with bone metastases. The second patient had a large clinically nonfunctioning metastasizing

Fig. 3  A and B, Electron micrograph of the pretreatment tumor demonstrates small undifferentiated tumor cells possessing few minute secretory granules, but no markers of PRL cell differentiation (A). Part B depicts 2 intermediate cells surrounded by aggregates of neuropil. The large cells possess considerable quantities of RER and sparse small secretory granules (original magnification ×7500).
producing tumors. In both patients, temozolomide medi-
cation caused impressive improvement. The patient of Zhu
et al [5] was a 61-year-old man with a PRL-secreting
pituitary carcinoma. Temozolomide administration to this
patient decreased blood PRL levels, caused clinical im-
provement, and significant tumor shrinkage. Zhu et al [5]
recommended temozolomide treatment as a novel approach
in patients with pituitary carcinoma.

In our case, after temozolomide administration, clinical
improvement was obvious and blood PRL levels markedly
decreased [6] similar to the cases of Fadul et al [4] and Zhu et al
[5]. However, our publication is the first that reports the
morphological findings and compares the changes in the
tumor before and after temozolomide therapy. The presence of hemorrhages, necrosis, fibrosis, and shrinkage in the tumor confirmed the antitumoral effect of temozolo-
mide. The study of prognostic indicators also showed that
temozolomide significantly decreased tumor cell proliferation.

The most intriguing finding was the presence of apparent
neuronal transformation in the tumor removed after treat-
ment with temozolomide. No neural elements were identi-
fied in the pretreated specimen. Neuronal transformation
that was unexpected in our case was described in several
pituitary tumor types, including pituitary adenomas, mainly GH-
producing tumors [13,14]. The causative mechanisms for
this transformation are unknown. In pituitary cells, exposure
to nerve growth factor resulted in neuronal transformation
[15]. It is not clear whether the process represents differen-
tiation, which slows tumor growth. Temozolomide may act similarly to nerve growth factor and cause neuronal
metaplasia, that is, transformation of pituitary tumor cells to
nerve cells.

Our work is based on the study of 1 single case only.
Thus, no definitive conclusions can be drawn. Despite
this limitation, the encouraging results support the view
that if other options fail, temozolomide should be adminis-
tered to patients with large, rapidly growing, invasive,
and aggressive PRL-secreting pituitary tumors. Our prelimi-
ary findings did not provide an answer whether other
pituitary tumor types would respond to temozolomide
therapy as well.

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