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Impact of temozolomide on gonadal function in patients with primary malignant brain tumors

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Abstract

Background—Cumulative exposure to alkylating agents may produce impaired reproductive function. Temozolomide is an alkylating agent approved for treating malignant gliomas.

Objective—A pilot study was undertaken to investigate the effects of temozolomide on semen integrity in men with newly diagnosed or recurrent malignant gliomas.

Methods—Eligible patients had no known fertility problems or impotence. Comprehensive semen analysis and serum sex hormones were obtained at baseline and following 3 and at least 6 months of temozolomide.

Results—Thirteen men were recruited. Mean age was 42 years (28–58). Three had recurrent and 10 newly diagnosed malignant glioma. Four were unable to ejaculate or were azoospermic at baseline. Four provided samples at baseline and after at least 6 months of temozolomide. Five were unable to complete the study. Two of four patients with paired baseline and 6-month samples received 6 months of standard monthly temozolomide. Two patients received standard radiation

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and concurrent temozolomide followed by adjuvant temozolomide. At 6 months, three of these four patients demonstrated low sperm motility (two low at baseline); three had abnormally low percent normal forms (one abnormal at baseline); two developed abnormally low sperm density. Sex hormone values were normal in all four patients at all time points.

Conclusion—Changes in semen analysis parameters following 6 months of temozolomide were observed. The small sample size precludes any firm conclusions regarding the importance and duration of these findings and their relation to temozolomide exposure. With validation in a larger study, these results may have important implications for counseling prior to initiation of temozolomide therapy in these patients.

Keywords

Malignant glioma; temozolomide; fertility

Introduction

Young adults with malignant cancers such as leukemia, lymphomas, and germ cell tumors are increasingly being cured with the use of combination chemotherapy. Moreover, survival of incurable cancers, such as malignant primary central nervous system tumors, is improving. The success of treatment with chemotherapeutic agents that are toxic to gonadal function has made infertility a significant concern for cancer survivors. As such, the American Society of Clinical Oncology has recognized the potential long-term physical and emotional distress caused by the use of chemotherapeutic agents and has issued guidelines on the risks of chemotherapy on survivorship.¹ Chemotherapeutic agents, particularly the alkylating agents and platinum drugs, have long been shown to have the potential to induce prolonged azoospermia in patients with Hodgkin's disease, non-Hodgkin's lymphoma, bone, and soft tissue sarcomas, testicular cancer, and leukemia.²⁻⁴ Among the germ cells which are within the seminiferous epithelium, the differentiating spermatogonia proliferate most actively and are extremely susceptible to cytotoxic chemotherapy agents.^{2,5-8} Male infertility rates vary between 28% and 63% in patients treated for newly diagnosed germ cell tumors.^{9,10} The induced azoospermia can be either temporary or prolonged, depending on the ability of surviving stem spermatogonia to proliferate, differentiate, and produce spermatozoa, which in turn depends on the duration of exposure to the cytotoxic agent and dose.^{6,11,12} As single agents, the alkylating drugs have been widely recognized to produce damage to the seminiferous epithelium. Azoospermia has been well documented in patients receiving various alkylating agents with the most toxic drugs including busulfan, melphalan,¹³ cyclophosphamide,^{14,15} and procarbazine.^{4,5,16}

Temozolomide (TMZ) is an oral imidazotetrazine second-generation alkylating agent with schedule-dependent antitumor activity against highly resistant malignancies including high-grade glioma. This novel oral alkylating agent causes methylation at the O⁶ position of guanine and alkylation at the N⁷ position. In clinical studies, TMZ has been shown to have reproducible linear pharmacokinetics with approximately 100% oral bioavailability and modest non-cumulative myelosuppression that is rapidly reversible.¹⁷ This agent has been approved in the United States for treatment of adult patients with refractory anaplastic astrocytoma and in conjunction with external beam radiation therapy for patients with newly diagnosed glioblastoma multiforme.

Reproductive function studies have not been conducted with TMZ in patients with malignant glioma (MG). Multi-cycle toxicology studies in rats and dogs have demonstrated testicular toxicity at doses of 50 mg/m² in rats and 125mg/m² in dogs which is 1/4 and 5/8, respectively, of the maximum recommended human dose based on body surface area.^{8,18} A single case report has recorded a patient with maintenance of fertility following treatment of

a high-grade astrocytoma with TMZ.¹⁹ While the risk of infertility following TMZ treatment has not been characterized, dacarbazine, with which TMZ shares the active metabolite monomethyl triazenoimidazole carboxamide (MTIC), has been shown to induce temporary reductions in sperm counts.²⁰ Furthermore, dacarbazine has been shown in at least one case series to have additive risk of infertility with prolonged use.²¹ Given that MTIC is the active metabolite of both TMZ and dacarbazine, there is reason to hypothesize that TMZ may have similar effects on fertility. As a result of the current standard use of TMZ as first-line therapy for MG, patients routinely receive 6 weeks of daily TMZ (75 mg/m²/d) along with radiation therapy followed by 6–12 months of adjuvant TMZ (150–200 mg/m²/d × 5 days each month). In this study, gonadal toxicity was explored in a series of male patients undergoing TMZ therapy for treatment of MG.¹⁸

Methods

A prospective pilot study was undertaken at a single comprehensive cancer center in the Southeastern United States to evaluate the gonadal side effects of TMZ therapy in adult patients with primary MGs. Eligible patients included adult male patients aged 18–60 years with a Karnofsky performance ≥ 60 and a histologically proven primary malignant brain tumor. Eligibility was not restricted to newly diagnosed patients, as patients with progressive disease or recurrent disease following prior radiation therapy were evaluated. Exclusion criteria included patients with a known inability to ejaculate, prior vasectomy or a known history of abnormal sperm motility and/or morphology. This study was approved by the institutional review board and all patients provided written informed consent prior to participating in study procedures.

Enrolled patients with a history of cranial irradiation received standard monthly TMZ at an oral dose of 150–200 mg/m²/day for 5 days on a 4-week cycle. Patients with no history of previous radiation and/or chemotherapy received concurrent external beam radiation therapy (approximately 6000 cGy) and daily TMZ 75 mg/m²/day followed by adjuvant monthly TMZ at 150–200 mg/m²/day for 5 days repeated every 28 days.

Sperm sample collection and semen analyses were conducted at a CLIA-certified Reproductive Medicine laboratory. Analysis included semen volume, viscosity, pH, forward progression, total count, total motile count, percent motility based on WHO fourth manual, presence of round cells, agglutination, and morphology. Enrolled patients underwent semen analysis prior to initiation of TMZ therapy and then at 3 and 6 months intervals. For patients who discontinued therapy prior to 6 months from initiation, a final analysis was performed at the time of discontinuation provided that they received at least one cycle of TMZ following the prior semen analysis. Serum studies including testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were performed at baseline and at each follow up interval.

All statistical analyses were performed with SAS Proc Mixed and SAS Proc Genmed programs. Descriptive statistics were calculated for the patient population. Categorical variables, such as forward progression, were analyzed using a McNemar test. Continuous variables, such as total motile count, were analyzed using paired *t*-test. Likelihood estimates for repeated measures were calculated using several computation methods including Newton–Raphson, Fisher scoring, and generalized expectation–maximization algorithms.

Results

Thirteen men were recruited for the study (Table 1). Mean age was 42 years (28–58). Three had recurrent MG, two with recurrent anaplastic astrocytoma and one with anaplastic

oligodendroglioma. Ten had newly diagnosed MG, four with anaplastic astrocytoma, three with GBM, one with gliosarcoma, one with oligodendroglioma, and one with astrocytoma. Three of the 13 patients had previously undergone brain radiation (external beam with 54–59.4 Gy). One of these had undergone prior chemotherapy with vincristine.

Four men were unable to ejaculate or were azoospermic at baseline (Table 2). Of the remaining nine patients, four provided samples at baseline and 6 months (except one patient whose follow up sample was collected following 12 months of TMZ chemotherapy). Reasons for non-compliance included clinical deterioration in one or modification of chemotherapy regimen in four. Of the nine viable baseline samples, 100% had normal sperm density and pH while 78% had normal sperm forms (>30%) and motility (>50%).

Two of four patients with paired baseline and 6 month samples were previously treated with brain irradiation and thus received 6 months of standard monthly TMZ, while two patients with newly diagnosed disease received standard concurrent chemoradiotherapy and adjuvant TMZ. Three of these four patients demonstrated low sperm motility at follow up, two of which were low at baseline (Table 3). Three of these patients had abnormally low percent normal forms at follow up, one of which was abnormal at baseline. Two patients developed an abnormally low sperm density at follow up. Testosterone, LH, and FSH levels at baseline and follow up were within the normal range in all four of these patients (Table 4). In all patients, average testosterone at baseline and follow up was 433 and 503 ng/dL, respectively (normal 270–1194 ng/dL). Average LH and FSH were 4.3 and 5.0 mIU/mL at baseline and 6.1 and 6.7 at follow up. No significant difference was found between sex hormone values at baseline and follow up in all patients ($p=0.93, 0.48, 0.059$ for testosterone, LH, and FSH, respectively).

Discussion

Patients with MG have varying degrees of reproductive function. The findings in this study support a variable effect on gonadal and semen function following initiation of TMZ therapy with persisting baseline dysfunction in some patients and deleterious effects in others. Overall, this study was difficult to accomplish and further study is required.

Five of nine men enrolled in this study had less than normal sperm motility at baseline and it generally remained below normal during treatment with TMZ. Two of these patients (patients 1 and 5) with baseline impairment in motility experienced persisting dysfunction during TMZ therapy. Two patients developed semen dysfunction (patients 2 and 3) following initiation and continued treatment with TMZ. While prior studies have demonstrated that a variety of chemotherapeutic agents can result in transient reductions in sperm count lasting 2–3 months, more toxic regimens can result in prolonged azoospermia as a result of damage to the very sensitive differentiating spermatogonia. Recovery depends on the survival of stem spermatogonia and their ability to differentiate. None of the patients with paired baseline and follow up samples had received chemotherapy prior to initiation of TMZ and all continued TMZ throughout the duration of this study. Patients 1 and 5 had received prior cranial irradiation while patients 2 and 3 received concurrent cranial irradiation during the initial period of study. Whether this had any differential effect on semen function between these patients is unknown. Patient 3 did receive intratumoral Gliadel wafers at the time of surgical resection which is a local therapy with minimal systemic toxicities. The wafer's effect on gonadal function is unknown. These findings raise the question of a possible alkylater affect on gonadal function in selected patients; although, no firm conclusions can be made. Anecdotally, we have cared for women who have successfully given birth following TMZ therapy and there is a single report of a man who conceived a child following TMZ treatment.¹⁹ Further study is required, but if replicated,

these observations may have important implications for physicians and pharmacists who educate and counsel patients on the important short- and long-term side effects of this medication.

Failure to ejaculate or azoospermia prior to initiation of TMZ therapy is also an interesting finding in this population and likely reflects a multi-factorial etiology relating to a variety of host-specific, tumor-specific, or prior treatment related factors. Systemic malignancies have been proposed to influence gonadal function through a variety of hormonal alterations and metabolic conditions.²² It has been suggested that reproductive hormones might be low in cancer patients as a result of systemic stress or downregulation by tumor-producing hormones such as B-human chorionic gonadotropins.²³ Cytokines released by malignant tumor cells as well as anorexia, fever, and pain can all worsen semen quality and affect gonadal function and/or sperm quality. In central nervous system tumors specifically, the hypothalamus and/or pituitary gland function may be impaired by tumor cell invasion, regional hormonal alterations, or damage from radiation therapy.²⁴ Cranial irradiation is known to affect fertility via disruption of the hypothalamic–pituitary–gonadal axis. Patients with cranial irradiation at doses of 35–45 Gy have been shown to subsequently develop deficiencies in gonadotrophins which can result in reduction of sex hormone concentrations including hypothalamic gonadotropin-releasing hormone.²⁵ In the MG patient population, this finding suggests a critical need for early counseling and discussion of the potential implications of concurrent chemoradiotherapy. In this population, average baseline sex hormone levels were within the normal ranges suggesting that the etiology of abnormal sperm and semen analyses in these patients may be unrelated to systemic hormonal levels.

This study has several important limitations. The small sample size limits the power of this study to provide conclusive causal evidence of TMZ induced sperm and semen abnormalities; much larger patient numbers would be needed for future confirmatory studies. A longer duration of follow up would allow further stratification of gonadal dysfunction into acute, subacute, and long-term toxicities. Investigation of additional serum markers including analysis of inhibin B and other potential markers of gonadal toxicity are potential options for future research in this area. Inhibin B is a glycoprotein excreted mainly from Sertoli cells in males which functions as a feedback regulator of FSH secretion from the pituitary. Wallace et al.²⁶ have published data which suggests that gonadotoxic chemotherapy is associated with reductions in inhibin B concentrations in male adults. This study was difficult to accomplish. Failure to ejaculate or azoospermia provided significant barriers to specimen collection at baseline and follow up. Several patients were unable to continue the study due to progression of disease, subsequent adjustments in chemotherapeutic regimens, and other clinical factors. Further study will require a larger sample size to accommodate these limitations.

Conclusion

In patients with MG, the high median age at diagnosis, the aggressive natural history of the disease, and the poor overall prognosis all impact reproductive capabilities. As mortality from MGs improves, particularly with more widespread use of TMZ in the treatment of glioblastoma multiforme and other lower grade brain tumors, gonadal toxicity and fertility will become an increasingly important consideration and potential toxicity of therapy. With the availability of assisted reproduction such as in vitro fertilization, intracytoplasmic sperm injection (a microinjection procedure for overcoming intractable male factor infertility), semen cryopreservation, and other technologies, severe deficits in semen quality can often be overcome. This article pilots an important area of TMZ research and highlights a relevant area for future study. Additional information regarding potential gonadal toxicity in these

patients will help inform discussions between clinicians, pharmacists, and patients regarding important reproductive considerations prior to and during initiation of therapy.

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Demographics

Table 1

Patient no.	Age	Diagnosis	Prior Tx	Current delivered Tx
1	52	Recurrent AA	XRT	TMZ (200 mg/m ² × six cycles)
2	33	AA	–	XRT + TMZ (TMZ 200 mg/m ² × six cycles)
3	47	Gliosarcoma	–	XRT + Gliadel + TMZ (TMZ 200 mg/m ² × six cycles)
4	32	AA	–	XRT + TMZ (TMZ 200 mg/m ² × six cycles)
5	30	Recurrent AA	XRT	TMZ (TMZ 200 mg/m ² × six cycles)
6	42	A	–	XRT + TMZ (TMZ 200 mg/m ² × one cycle)
7	28	GBM	–	XRT + TMZ (TMZ 200 mg/m ² × four cycles)
8	35	AA	–	XRT + TMZ (TMZ 200 mg/m ² × five cycles)
9	44	O	–	XRT + TMZ (TMZ 200 mg/m ² × seven cycles)
10	47	GBM	–	XRT + TMZ → poly ICLC + TMZ (TMZ 200 mg/m ² × one cycle)
11	58	GBM	–	XRT + arsenic → TMZ (TMZ 200 mg/m ² × one cycle)
12	58	AA	–	XRT + TMZ (TMZ 200 mg/m ² × six cycles)
13	44	Recurrent AO	XRT and PCV	TMZ (TMZ 200 mg/m ² × four cycles)

AA: anaplastic astrocytoma; A: astrocytoma; GBM: glioblastoma multiforme; O: oligodendroglioma; AO: anaplastic oligodendroglioma; XRT: radiation therapy; and TMZ: temozolomide.

Demographics for all patients including age, diagnosis, prior chemotherapy or radiation therapy and chemotherapy or radiotherapy delivered during the study.

Table 2

Semen samples obtained.

Patient no.	Semen samples obtained		
	Baseline	3 months	6 months
1	+	+	+
2	+	Declined	+
3	+	Unable	+*
4	+	Unable	Off-study
5	+	+	+
6	+	Chemo dc'd	Off-study
7	+	Unable	Died
8	+	Unable	Off-study
9	+	Declined	Off-study
10	Unable	Off-study	Off-study
11	Unable	Off-study	Off-study
12	Azoospermic	–	+
13	Azoospermic	Azoospermic	Progressed

Baseline, 3-month, and 6-month semen samples for the four patients with baseline and 6-month samples (patients 1–3, 5); for the five patients who were removed from study secondary to clinical deterioration or modification of chemotherapy (patients 4, 6–9); and for the four patients who were azoospermic or unable to ejaculate at baseline (patients 10–13).

* Follow up sample was available only at 12 months.

Table 3

Semen analysis for patients with baseline and at least 6-month follow up.

Patient no.	pH (7.5–9.0)	Density (20–300 × 10 ⁶)	% Motility (50%)	% Normal forms (30%)
1	Baseline	24	1	11
	3 months	20	30	40
	6 months	11	4	14
2	Baseline	44	41	68
	6months	0.3	42	7
3	Baseline	24	50	50
	12 months	58	12	14
5	Baseline	76	39	33
	3 months	20	40	44
	6 months	72	22	53

TMZ: temozolomide.

Semen characteristics including pH, density, percent motility, and percent normal forms for the four patients with paired baseline and at least 6-month follow up demonstrating reduced motility and reduced normal forms in three of the four patients at follow up. Follow up sample was available only at 12-month follow up for patient 3 who continued TMZ chemotherapy throughout this duration.

Table 4

Sex hormone values for patients followed for at least 6 months.

Patient no.	Normal	Testosterone	LH	FSH
		(270–1194 ng/dL)	(0.910.6 mIU/mL)	(2.0–17.7 mIU/mL)
1	Baseline	–	–	–
	3 months	457	4.8	8.2
	6 months	336	3.5	7.2
2	Baseline	608	6.8	5.6
	6months	546	7.2	7.2
3	Baseline	160	3.3	3.6
	12 months	160	5.4	7.3
5	Baseline	776	4.3	1.5
	6 months	340	–	2.0

LH: luteinizing hormone; FSH: follicle-stimulating hormone.

Sex hormone values including total testosterone, LH, and FSH for the four patients with paired baseline and at least 6-month follow up demonstrating normal sex hormone characteristics at baseline and follow up.