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ORIGINAL ARTICLE

Hematologic adverse events associated with temozolomide

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Abstract
Purpose Temozolomide (TMZ) is a widely used oral alkylating agent that has been associated with the development of severe hematologic adverse events (HAEs). Limited clinical information about HAEs is available.
Methods We searched the FDA MedWatch database for TMZ and obtained all MedWatch reports on TMZ submitted to the FDA from November 1, 1997 to September 3, 2008. We defined major HAEs, namely agranulocytosis, aplasia, aplastic anemia (AA), leukemia (various), myelodysplastic syndrome (MDS), and lymphoma, and several minor HAEs.
Results A total of 5,127 reports on 3,490 patients were submitted to MedWatch. Among these, we identified 112 cases of major HAEs. Of the 44 reported deaths, the major HAE was considered the cause in 32 cases. The median duration of TMZ treatment was 6 weeks [0.5–108 weeks]. Seventy-six cases of AA or aplasia and 17 cases of leukemia represented the most common major HAE. Important minor HAEs were bone marrow failure and pancytopenia/pancytopenia-like with 325 combined cases; these reports are clinically similar to aplastic anemia.
Conclusion The hematologic toxicity profile of TMZ differs from that of other alkylating agents. TMZ HAEs are emerging as significant concerns. Among alkylating agents, AA appears unique to TMZ, and the high rate warrants disclosure of patients. The duration of TMZ exposure prior to the development of AA may be quite short. The risk of AML/MDS is low, but the length of follow-up is insufficient to assess the true risk.

Keywords Temozolomide · Secondary leukemia · Aplastic anemia · Alkylating chemotherapy · Brain tumor

Introduction

Temozolomide (TMZ) has been commercially available in the United States since 1999. It is used extensively in the treatment of newly diagnosed glioblastoma and refractory anaplastic astrocytoma. In addition, TMZ is increasingly being used in the treatment of lower-grade gliomas and for durations longer than those that have been well studied [35]. In 2006, the first report of aplastic anemia (AA) associated with TMZ was published [34]. Subsequently, multiple reports of AA, myelodysplastic syndrome (MDS), and leukemia, as well as severe myelosuppression, have appeared [6, 7, 9, 14, 20, 24–26, 30, 32]. In 2007, the FDA issued a Drug Safety Newsletter, in which they described 18 cases of AA in patients treated with TMZ. These cases were reported to the FDA during the time period August 11, 1999, to November 3, 2006 [12]. Schering-Plough

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Table 1 Major hematologic adverse events

	Cases	Age				Gender			Death due to HAE
	N	*N*	Mean	Median	Range	*N*	Male	Female	*N*
Agranulocytosis	7	5	51	54	51–70	6	1	5	2
Aplasia	37	35	54	59	4–80	37	11	26	11
Aplastic anemia	39	32	50	54	4–74	38	16	22	11
Myelodysplastic syndrome	7	6	52	63	6–74	6	5	1	2
Lymphoma	5	5	47	50	25–73	5	2	3	2
Leukemias	17	12	48	48	14–66	16	6	10	4
Bone marrow failure*	97	79	55	57.5	3.5–79	92	40	52	20
All pancytopenia*	363	314	57.6	61	2–84	352	145	207	83
Reported pancytopenia*	228	201	58.1	62	2–84	217	84	133	56
Pancytopenia-like*	135	135	56.9	60	84–113	135	61	74	27

* Initially considered minor hematologic adverse events

estimates the incidence of AA to be 10.22/100,000 patients exposed to TMZ [35]. We reviewed and characterized TMZ-associated hematologic adverse events (HAEs) reported through the MedWatch reporting system.

Methods

We obtained the FDA MedWatch database for all TMZ-related adverse events (AE) reported between November 1, 1997 and September 4, 2008, using the Freedom of Information Act. The database contained 5,127 reports, including initial and follow-up reports. The reports were sorted and those describing the same patient were identified where possible by comparing report numbers, age, gender, concomitant medications, and TMZ dose. After sorting, we counted approximately 3,400 cases (patients) for whom reports were submitted. For the purpose of this analysis, we combined what appeared to be variations of the same HAE term. Next, we classified the HAEs as major or minor, based on the limited clinical descriptions provided (Table 1). If more than one HAE term appeared in a report, the case was counted under only one term. Pancytopenia was frequently reported in combination with thrombocytopenia and/or neutropenia and/or anemia, and we initially classified pancytopenia as a minor HAE. If a report included two or more of thrombocytopenia, neutropenia, or anemia and no additional major or minor HAE (and did not include the term pancytopenia), the case was labeled pancytopenia-like. After the initial identification and analysis of 112 cases of major HAE, we obtained the actual MedWatch reports for these cases in order to further characterize the events. Full review of these cases led to a modification in analysis. We subsequently reviewed all reports for the minor HAEs, i.e., bone marrow failure, pancytopenia, and pancytopenia-like, and reclassified them as major HAE. The database was initially reviewed by two reviewers. The actual reports were reviewed by one of the reviewers.

Results

We initially identified 112 cases describing major HAEs (Table 1). Major HAEs include agranulocytosis, aplasia, AA, leukemia (various), MDS, and lymphoma. Information regarding age was available for 95 major cases (85%). Median age (years) was 54 for AA and agranulocytosis, 59 for aplasia, 48 for all leukemias, 63 for MDS, and 50 for lymphoma. Gender was reported for 108 major cases (96%), with 65 major cases in women and 43 major cases in men. Most patients received TMZ for CNS tumors (76%). Forty-four deaths were reported among the 112 major cases. The reporter attributed death to the HAE in 32 cases. The median duration of TMZ treatment was 6 weeks [0.5–108 weeks] for all major cases combined, while the median onset of clinical findings (e.g., neutropenia) was 4 weeks [0.7–208 weeks]. Fourteen cases reported the use of other alkylating agents, and 6 of those cases reported leukemia. Radiation therapy was reported in 40 cases, mostly in AA and aplasia. Information documenting recovery from HAE was reported in 14 cases. Thus, the outcome of HAE is not known in at least 54 cases (112−[number of deaths + number recovered]). Most of the reports were filed by a health professional. Information regarding concomitant medications and other anticancer treatments was not consistently reported and varied between cases (Table 2).

Aplastic anemia was the most frequently reported HAE with 39 cases and was the cause of death in 11 patients.

Table 2 Summary of HAEs

HAE	*N*	Median duration of treatment in weeks (range)	Median onset of symptoms in weeks (range)	Alkylating agents (*n*)	RT (*n*)	Death reported as related to HAE (*n*)	Recovery reported (*n*)
All events	112	6 weeks (0.5–108)	4 weeks (0.7–208)	14	40	31	14
Leukemia	17	28 weeks (4–54)	29 weeks (3–208)	6	7	3	1
Aplastic anemia	39	6 weeks (0.6–68)	4 weeks (1–24)	3	17	11	4
Aplasia	37	4 weeks (0.6–24)	2.4 weeks (0.7–36)	3	10	11	7
Lymphoma	5	51 weeks (20–108)	16 weeks (4–44)	1	3	2	1
Agranulocytosis	7	4 weeks (2–24)	4 weeks (1–24)	0	1	2	2
MDS	7	50 weeks (7–104)	16 weeks (0.5–208)	1	3	3	Unknown
Bone marrow failure	97	3.6 weeks (0.3–104)	3 weeks (0.1–130)	5	29	20	24
Pancytopenia	363	2.7 weeks (0.1–104)	0.9 weeks (0–7)	23	111	83	135
Reported Pancytopenia	228	3 weeks (0.1–104)	0.6 weeks (0–4)	10	59	56	87
Pancytopenia-like	135	1.8 weeks (0.3–40)	1.3 weeks (0–7)	13	52	27	48

Most deaths were due to infection. The median age of the patients was 54 [4–74]; 22 women and 16 men were affected. The median duration of TMZ therapy was 6 weeks [0.6–68], while the median onset of clinical findings was 4 weeks [1–24]. Sixteen patients received radiation therapy, and three received other alkylating agents in addition to TMZ.

Aplasia was reported 37 times and also caused 11 deaths. Twenty-six women and eleven men had a median age of 59 years [4–80]. Three patients had received alkylating agents, and ten had been treated with radiation. Seven patients were reported as recovered. Median duration of treatment with TMZ was 4 weeks [0.6–24], with the onset of clinical findings at 2.4 weeks [0.7–36].

Agranulocytosis occurred in seven cases. The median age was 54 years [51–70]. The median duration of TMZ was 4 weeks [2–24]. The onset of HAE was also 4 weeks [1–24]. One patient was treated with radiation. Recovery from agranulocytosis was noted in two patients.

Seventeen cases of leukemia were reported. Three cases described progression from MDS. From the available data, median age was 48 [14–66]. Ten women and six men were affected. Seven patients received radiation therapy, and six patients received other alkylating agents before or after TMZ. Median duration of TMZ therapy was 28 weeks [4–54 weeks], and median onset of HAE was 29 weeks [3–208 weeks]. There were three HAE-related deaths.

Myelodysplastic syndrome (without leukemia) was reported seven times. Five men and one woman were described with a median age of 63 years [6–74]. The median duration of TMZ was 50 weeks [7–104], and clinical findings were seen at a median of 16 weeks [0.5–208]. One patient had received an alkylating agent at some point, and three patients were treated with radiation. Two deaths resulted from MDS.

There were five reports of lymphoma**,** three in women, and two in men. Lymphoma was fatal in two patients. Patients were treated with TMZ for a median of 51 weeks [20–108]. HAE appeared after a median of 16 weeks [4–44]. Three patients had been treated with radiation, and one had also received alkylating agents.

Based on the reports of major HAEs, ten patients received TMZ for more than 6 months. Three patients treated with adjuvant TMZ for glioblastoma received treatment beyond the recommended duration. Two of these cases developed lymphoma, and one developed MDS. The other indications for prolonged TMZ were anaplastic astrocytoma, glioma, and oligodendrioglioma. Three patients developed leukemia, two MDS, and one aplastic anemia (Table 3).

Other HAE

We subsequently evaluated all reports of bone marrow failure, pancytopenia (reported), and pancytopenia-like. We identified 97 cases labeled as bone marrow failure. The median age in these patients was 57.5 years. Fifty-two patients were women, and 40 were men. Most of the patients were treated with temozolomide for brain tumors. The median duration of treatment with TMZ was 3.6 weeks, and the median onset of clinical findings was 3 weeks for patients with bone marrow failure.

Pancytopenia was reported in 228 patients. It affected women more than men (133 vs. 84). The median age of patients developing pancytopenia was 62 years. Fifty-six

Table 3 Indications of treatment with temozolomide

Indication for temozolomide	Six major adverse events		Bone marrow failure		Pancytopenia (all)		Reported pancytopenia	
	$N = 112$	%	$N = 97$	%	$N = 363$	%	$N = 228$	%
CNS tumor	85	76	76	78	262	72	163	71
Melanoma	3	3	5	5	46	13	28	12
Lymphoma	3	3	2	2	3	1	2	1
Brain metastasis	3	3	2	2	5	1	0	0
Ependymoma	2	2	1	1	1	0.3	0	0
Medulloblastoma	1	1	3	3	1	0.3	1	0.4
Neuroblastoma	1	1	–	–	2	0.6	0	0
Other	2	2	–	–	24	7	14	6
Not reported	12	11	8	8	21	6	20	9

deaths were reported as related to pancytopenia or its complications. The median duration of TMZ treatment was less than 3 weeks. Most of the cases were diagnosed within a week of the end of treatment and in some cases, during concurrent TMZ/radiation (59 patients).

The term pancytopenia generally refers to at least two cell lineages affected, i.e., anemia and/or neutropenia and/or thrombocytopenia. We identified 135 cases that were not labeled as pancytopenia, but included at least two of the three cytopenias (i.e., pancytopenia-like). The median age of patients who developed the pancytopenia-like HAE was 60 years, and again, female gender predominated. Twenty-seven deaths were associated with the pancytopenia-like HAE.

Discussion

Our analysis demonstrates that the spectrum of HAEs associated with TMZ encompasses severe myelosuppression and leukemia, as well as AA. The cytotoxic effect of TMZ is due to the generation of O^6-methylguanine (O^6-meG) DNA adducts. Methylguanine methyltransferase (MGMT), when present, removes the adduct and reduces clinical activity. Cell death occurs when the mismatch repair (MMR) system recognizes abnormal base pairing and attempts to correct it. Bone marrow precursors generally have low levels of MGMT [16]. MGMT activity also decreases subsequent to DNA damage (e.g., after TMZ), and this might further predispose to bone marrow toxicity. In addition, some polymorphisms of *MGMT* may be associated with an increased risk of bone marrow toxicity [4, 10, 16, 17, 21, 35].

O^6-meG lesions are also mutagenic [10, 21, 29, 36]. MMR-deficient cells survive with a mutation, leading to increased genetic instability and predisposing to leukemogenesis. Defective MMR has been identified in many cases of alkylator-induced AML, and polymorphisms in the MMR pathway may increase the risk of secondary malignancy following treatment with TMZ [36].

Temozolomide and myelosuppression

TMZ is usually considered well tolerated, with relatively mild myelosuppression. The "reference rate" for TMZ hematologic toxicity is based on the 2005 paper of Stupp et al. [31]. In this study of concurrent TMZ/radiation followed by adjuvant TMZ, grade 3 or 4 thrombocytopenia was seen in 3% of 287 patients during concurrent therapy and in 11% during adjuvant TMZ. Grade 3 or 4 neutropenia was described in 4% of patients during both concurrent therapy and adjuvant TMZ. Other reports suggest that the rate of myelosuppression may be higher, accounting for a substantial number of treatment discontinuations [15].

Some investigators have tried to identify predictors of TMZ hematologic toxicity. Sabharwal et al. [28] noted that low pretreatment MGMT in peripheral blood mononuclear cells was associated with thrombocytopenia and neutropenia after one cycle of TMZ or dacarbazine. Depletion of MGMT during TMZ was accompanied by increases in myelosuppression. In contrast, a recent abstract described the methylation status of the *MGMT* promoter in 13 of 25 patients who developed grade 3–4 myelosuppression with TMZ [33]. *MGMT* promoter was methylated in five patients in whom the median time to recovery was 12 days (range 7–122 days). *MGMT* promoter was unmethylated in eight patients in whom the median time to recovery was 35 days (range 25–98). This difference in time to recovery from myelosuppression was not statistically significant, although the direction is the opposite of what would be expected. The abstract does not specify the cells in which *MGMT* methylation was tested.

Nagane et al. [25] also analyzed methylation status of the *MGMT* promoter in a patient who developed prolonged pancytopenia during concurrent TMZ/radiation. The tumor

specimen demonstrated methylation of the *MGMT* promoter, with no detectable MGMT protein. Despite the methylation status, tumor progression was noted within 10 weeks. Analysis of peripheral blood leukocytes and bone marrow cells at the time of partial recovery of peripheral blood counts demonstrated unmethylated *MGMT* promoter. This too is opposite of what would be expected. However, the methylation status was only determined once the bone marrow had begun to recover, 3 months removed from TMZ.

Armstrong et al. [1] identified clinical factors associated with myelosuppression with the first cycle of TMZ and proposed a model to predict the likelihood of myelosuppression. The incidence of myelosuppression was higher in women than in men. This is consistent with our analysis. Clinical factors identified in men included BSA greater than 2 m^2, not being on steroids, and taking bowel medication (presumably laxatives). In women, clinical factors associated with the development of myelosuppression were no prior chemotherapy, baseline creatinine 1 mg/dl or greater, baseline platelet count less than 270,000/mm^3, BSA less than 2 m^2, being on analgesics (specific analgesics or analgesic classes not noted), and not being on medication for GERD. These factors could not be evaluated in our data [1]. The relevance of these clinical factors is not readily apparent.

Polymorphisms in the *MGMT* gene could potentially alter repair capability and thus predispose to myelosuppression from TMZ. However, most of the polymorphic variants described to date do retain repair activity [11, 23]. Indeed, ongoing research is directed at achieving expression of the P140K mutant ($MGMT^{P140K}$) in bone marrow stem cells and progenitor cells, the goal being selective protection of hematopoietic cells. Polymorphic variants may have different effects, based on the amount of expression. Milsom et al. [23] found, in a mouse model, that very high overexpression of $MGMT^{P140K}$ had a negative effect on hematopoietic stem cell reconstitution in vivo and on cell proliferation in vitro.

Thus, TMZ shares a cytotoxic mechanism with dacarbazine and other methylating compounds, and distinct from the "traditional" alkylating agents. MGMT protects against TMZ cytotoxicity, and bone marrow precursors generally have low levels of MGMT relative to other cell populations. Furthermore, MGMT activity varies between individuals. Qualitative or quantitative differences in MGMT activity could account for varying degrees of myelosuppression.

Temozolomide and mutagenicity

TMZ mutagenicity might account for the growing number of cases of treatment-related AML (tAML) and MDS, especially in MMR-deficient cells. tAML is reported in 3–10% of patients who receive alkylating agents for Hodgkin lymphoma, non-Hodgkin lymphoma (NHL), ovarian cancer, breast cancer, and multiple myeloma [29]. "Traditional" alkylator-induced AML is often associated with the losses or deletions of chromosome 5 or 7 and often preceded by MDS. The classic picture includes the onset of AML at 5–7 years after aklylator therapy. The time between the development of MDS and the development of AML is variable, which may represent the time required for subsequent genetic events following a chromosomal alteration [19, 27, 29]. The "classic picture" of TMZ-associated AML/MDS is still emerging. All alkylating agents do not have the same leukemogenic potential. TMZ appears to be more mutagenic than some other alkylators. Geiger et al. [13] demonstrated a higher mutation rate in mouse bone marrow cells exposed to TMZ as compared to those exposed to cyclophosphamide. Mutation frequency increased twofold over baseline following cyclophosphamide and 22-fold over baseline with TMZ. Using a model of *Mgmt*−/− and *Mgmt*+/+ mice, Hansen et al. [18] also suggested that TMZ is more mutagenic than carmustine. If TMZ is indeed more mutagenic than other alkylators, this could translate into an increased likelihood of genetic instability and secondary malignancy. The fact that there have been less major HAEs reported with TMZ as compared with the traditional alkylators may be a result of the latter having been in use longer. In addition, the survival of patients with CNS tumors is generally shorter than the survival times in the often curable malignancies treated with the traditional agents.

Temozolomide and aplastic anemia

Aplastic anemia is not described in most of the literature reporting traditional alkylator-induced AML. AA is characterized by peripheral blood pancytopenia and a hypocellular marrow. There is no formal WHO definition of AA, and it is not clear whether AA is unique to TMZ or whether the cases in the literature and in the MedWatch database were arbitrarily labeled as such.

The reporting of AA seems to be somewhat unique to TMZ among the alkylating agents. It is possible that some cases of "aplastic anemia" might have progressed to MDS or AML after the time period covered in the MedWatch report. It is also possible that patients died due to underlying disease (i.e., CNS tumor) before the outcome of "aplastic anemia" could be determined. In our analysis, the median duration of TMZ treatment in the 39 cases of AA was only 6 weeks. This is similar to the median duration of TMZ treatment in 228 patients with "pancytopenia" (3 weeks) and 37 patients with "aplasia" (4 weeks). The

patient demographics and outcomes were also similar among these three diagnoses. The MedWatch cases of bone marrow failure, as well as those that we termed "pancytopenia-like", are also similar in terms of duration of TMZ therapy and demographics to the cases of AA. Furthermore, it would seem that in most of these cases, the term "myelosuppression" could easily have been substituted for the HAE. The MedWatch database includes reports from all over the world, and our analysis suggests that different adverse event terms are used to describe the same clinical picture.

The literature provides support for the notion that multiple event terms are describing the same clinical phenomenon. "Aplastic anemia" and "aplasia" are used interchangeably, as are "bone marrow failure" and "aplastic anemia" [8, 29]. Multiple terms are also used in the reports of TMZ-induced HAEs. Recent case reports of aplastic anemia describe "pancytopenia" and an "aplastic marrow" on bone marrow biopsy and "pancytopenia" with a "hypocellular" marrow with "trilineage hypoplasia" [14, 24]. In Gerber's series of 52 patients, two patients developed prolonged thrombocytopenia that lasted for more than 6 months [15]. In one of these patients, a bone marrow examination on day 127 demonstrated marked hypocellularity, and the patient was considered to have "aplastic anemia". And in the case reported by Nagane et al. [25], the patient developed "pancytopenia" during concurrent TMZ/radiation. Thrombocytopenia was noted on day 27 of treatment. Ten days later, the patient developed neutropenia, followed by anemia. Partial recovery was seen in about 3 months. The onset and course of cytopenias in this case are similar to the cases of "aplastic anemia", "aplasia", and "pancytopenia" in our analysis.

Conclusion

Our analysis demonstrates that marked bone marrow toxicity most often occurs early in treatment, compared with the events of leukemia or MDS. This suggests that there might be a genetic susceptibility, as yet undefined, to "expected" hematologic toxicity.

The quality of the MedWatch reports is a recognized limitation of our analysis. Cases with missing information could not be fully analyzed, and duplicate cases had to be identified by matching clinical and demographic information. Our analysis cannot provide an estimate of incidence, but only an awareness and relative frequency of these HAEs. Though having limitations, MedWatch is an important resource for drug safety analysis on post-approved therapies yielding useful safety concepts [2, 3, 5, 22].

In conclusion, TMZ-induced HAEs are emerging as a significant concern. The spectrum of MedWatch HAEs includes severe myelosuppression and pancytopenia that are clinically similar to aplastic anemia, as well as MDS and AML. This spectrum of HAEs is consistent with the cytotoxic and mutagenic properties of the O^6-meG lesion induced by TMZ. Recognition of the potential for these events is particularly important in light of the increasing use of more protracted TMZ regimens. The MedWatch database illustrates the growing number of cases of TMZ HAEs and also highlights the need for consistent terminology and improved characterization of these important events.

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