A Phase I and Pharmacokinetic Study of Temozolomide and Cisplatin in Patients with Advanced Solid Malignancies


ABSTRACT

Temozolomide (TMZ) is an oral imidazotetrazinone that is spontaneously converted to 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) at physiological pH. MTIC methylates DNA at the O6 position of guanine, although this lesion may be repaired by the enzyme O6-alkylguanine-DNA alkyltransferase (AGAT). In this study, TMZ was combined with cisplatin (CDDP), because both agents have single-agent activity against melanoma and other tumor types. Additionally, CDDP has been shown to inactivate AGAT, and subtherapeutic concentrations of CDDP have been shown to increase the sensitivity of leukemic blasts to TMZ. This Phase I study sought to determine the toxicities, recommended dose, and pharmacological profile of the TMZ/CDDP combination.

Patients were treated with oral TMZ daily for 5 consecutive days together with CDDP on day 1 (4 h after TMZ) every 4 weeks at the following TMZ (mg/m2)/CDDP (mg/m2) dose levels: 100/75, 150/75, 200/75, and 200/100. Plasma samples were obtained on days 1 and 2 to evaluate the pharmacokinetic parameters of TMZ alone and in combination with CDDP. Fifteen patients received a total of 44 courses of TMZ/CDDP. The principal toxicities of the regimen consisted of neutropenia, thrombocytopenia, nausea, and vomiting, which were intolerable in two of six new patients treated at the 200/100 mg/m2 dose level. Of five patients receiving 17 courses at the next lower dose level (200/75 mg/m2), none experienced dose-limiting toxicity. Antitumor activity was observed in patients with non-small cell lung cancer, squamous cell carcinoma of the tongue, and leiomyosarcoma of the uterus. Pharmacokinetic studies of TMZ revealed the following pertinent parameters (mean ± SD): time to maximum plasma concentration (Tmax) = 1.1 ± 0.6 h (day 1) and 1.7 ± 0.9 h (day 2); elimination half-life (T1/2) = 1.74 ± 0.22 h (day 1) and 2.35 ± 0.70 h (day 2); and clearance (CL/F) = 115 ± 27 ml/min/m2 (day 1) and 141 ± 109 ml/min/m2 (day 2). TMZ drug exposure, described by the area under the plasma concentration-time curve (AUC∞) and the maximum plasma concentration (Cmax), was similar on days 1 and 2.

On the basis of these results, the recommended doses for Phase II clinical trials are TMZ 200 mg/m2/day for 5 days with 75 mg/m2 CDDP on day 1, every 4 weeks. The addition of CDDP did not affect the tolerable dose of single-agent TMZ (200 mg/m2/day × 5 days), nor did it substantially alter the pharmacokinetic behavior of TMZ.

INTRODUCTION

The rationale for the development of TMZ3 (Fig. 1) is to increase the therapeutic index of the imidazotetrazinone alkylating agents (1). Like DTIC, the prototypical compound of this class, TMZ is a produg of MTIC (1–5), the putative active species that methylates DNA at the O6 position of guanine (6–13). DTIC requires hepatic p450-mediated metabolic activation to generate MTIC, which is associated with high interindividual variability (2, 14), whereas TMZ is converted to MTIC spontaneously, rapidly, and predictably at physiological pH (1–3). This more consistent generation of the active moiety after TMZ administration may result in substantially less interindividual variability in the generation of MTIC and hence greater predictability with respect to the toxicity and efficacy of this imidazotetrazinone.

In preclinical studies, TMZ has demonstrated antitumor activity against human lymphoblastoma, myeloid leukemia, Burkitt’s lymphoma, choriocarcinoma, astrocytoma, and lung.

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3 The abbreviations used are: TMZ, temozolomide; DTIC, dacarbazine; MTIC, 5-(3-methyltriazene-1-yl)methyl-4-carboxamide; AGAT, O6-alkylguanine-DNA alkyltransferase; DLT, dose-limiting toxicity; AUC, area under the plasma concentration-versus-time curve; CDDP, cisplatin; MTD, maximum tolerated dose; ECOG, Eastern Cooperative Oncology Group; ANC, absolute neutrophil count.
colorectal, and testicular carcinoma cell lines, with IC₅₀ values ranging from 10 to 718 µM (6, 15, 16). The activity of TMZ in vitro has been shown to be inversely proportional to the activity of the DNA repair enzyme AGAT (6–9), and certain cell lines are more sensitive to TMZ after AGAT inhibition (6, 7, 9–13). In the human tumor cloning assay, TMZ inhibits the growth of clonogenic cells from human tumors obtained from patients with melanoma, sarcoma, and breast, ovarian, lung, renal cell, colon, prostate, and pancreas carcinomas (17). In vivo, both i.p. and oral administration of TMZ prolong the survival of nude mice bearing murine TLX5 lymphoma (1). The effects on survival are schedule dependent, with greater efficacy noted with repetitive divided dose administration (e.g., daily for 5 days) compared with a single bolus administration (1). Additional studies have demonstrated appreciable cytoreduction in glioma, ependymoma, melanoma, ovarian, and colon cancer xenografts (18–20).

In Phase I clinical trials, the principal DLT of TMZ has been myelosuppression (21–25). Although nausea and vomiting were initially reported to be common, these effects have been mitigated by antiemetic medications, particularly serotonin antagonists (21–25). When administered daily for 5 days every 4 weeks, the recommended Phase II dose of TMZ has ranged from 125 to 225 mg/m²/day (21–24). Drug exposure, measured as the AUC, has increased linearly with dose, and drug clearance has been dose independent, with mean values ranging from 102 to 115 ml/min/m² (4, 21–24). Oral TMZ has also been reported to be nearly completely bioavailable (>99%; Refs. 4, 21). In the initial Phase I trial, there were no objective responses noted in 51 patients treated with a single dose of TMZ (21). In view of the schedule-dependent antitumor activity in preclinical studies, an additional 42 patients were treated with TMZ daily for 5 days every 4 weeks (21). On this schedule, there were five responses, including two complete responses in patients with melanoma (21). Additional studies have confirmed the promising activity of TMZ on the daily × 5 schedule in both melanoma (26) and high-grade astrocytoma (27–29), with response rates of 24 and 27–42%, respectively.

In this Phase I trial, oral TMZ was administered as a single oral dose daily for 5 days every 4 weeks with CDDP administered i.v. on day 1. The rationale for evaluating this regimen was, in part, the single-agent activity of both TMZ (21–23, 26) and CDDP (30) against melanoma, a relatively refractory tumor type. Furthermore, CDDP had been shown to reduce AGAT activity in vitro (31), suggesting that CDDP might enhance the antitumor activity of TMZ. This was supported by the demonstration of enhanced cytotoxicity in leukemic blasts treated with TMZ and CDDP at subtherapeutic concentrations (32). In addition, with nonoverlapping DLTs, the administration of TMZ and CDDP was anticipated to be feasible. Finally, even in the presence of CDDP-induced nephrotoxicity, the disposition of TMZ would not be expected to be affected, because TMZ is only minimally disposed of by renal mechanisms (5–7%; Refs. 4, 22, 23). The objectives of this Phase I and pharmacokinetic study were to: (a) describe the DLTs of TMZ administered as a single oral dose daily for 5 days every 4 weeks combined with CDDP as a 60-min infusion on day 1; (b) determine the MTD and recommended Phase II doses of TMZ and CDDP on this schedule; (c) characterize the pharmacokinetic behavior of TMZ in combination with CDDP; and (d) report any observed anticancer activity in patients with advanced solid malignancies.

PATIENTS AND METHODS

Patient Selection. Patients with histologically confirmed advanced solid malignancies refractory to standard therapy or for whom no effective therapy existed were candidates for this study. Other eligibility criteria included: (a) age ≥18 years; (b) ECOG performance status of ≤2 (ambulatory and capable of self care); (c) life expectancy >12 weeks; (d) no known brain metastases or primary central nervous system tumor; (e) no chemotherapy, radiotherapy, or biological therapy in the previous 4 weeks; (f) no prior therapy with mitomycin C or nitrosoureas; (g) no cumulative prior radiotherapy to fields containing >15% of the bone marrow reserve; (h) adequate hematopoietic (ANC ≥1500/µl and platelet count ≥100,000/ µl), hepatic (total serum bilirubin within the upper limit of normal and transaminases ≤2 times upper limit of normal), and renal (serum creatinine concentration ≤1.5 mg/dl) functions; (i) no frequent vomiting or medical condition that could interfere with the absorption of oral medications; and (j) no other coexisting medical problems of sufficient severity to prevent full compliance with the study. In addition, patients were ineligible for this study if they had received prior DTIC or more than three prior courses of platinum-based chemotherapy. Written informed consent was obtained according to federal and institutional guidelines.

Drug Dosage and Escalation. The starting doses were 100 mg/m² TMZ as a single oral dose daily on days 1–5 and 75 mg/m² CDDP i.v. over 1 h on day 1, 4 h after the administration of TMZ. Courses were administered at a minimum interval of 28 days. The dose of TMZ in this combination was escalated in each successive group of new patients until the previously determined MTD for single-agent TMZ (200 mg/m²/day) was reached (21). CDDP was then escalated from 75 to 100 mg/m². The following dose levels of TMZ (mg/m²/day)/CDDP (mg/m²) were planned: 100/75, 150/75, 200/75, and 200/100. It was planned to enroll one patient at the first dose level to ensure that myelosuppression was not more severe than anticipated from studies of single-agent TMZ. Thereafter, it was planned to enroll at least three patients at each dose level until DLT was reached. DLT was defined as any one of the following: (a) ANC <500/µl lasting for longer than 5 days or associated with fever; (b) platelet count <50,000/µl; (c) hemoglobin <6.5 g/dl; (d) serum creatinine >2.2 mg/dl, persisting for >2 weeks after the scheduled date of retreatment; and (e) nonhematological toxicity.
that was at least National Cancer Institute grade 3 in severity (excluding grade 3 nausea or vomiting and grade 3 fever in the absence of infection). If one DLT occurred, a maximum of six patients were treated at that dose level. The MTD level was defined as the highest dose level in which fewer than two of six new patients experienced DLT. Toxicities were graded according to the National Cancer Institute common toxicity criteria (33).

Patients who had not developed progressive disease at the time of reassessment and who had not experienced DLT were permitted to continue treatment with TMZ and CDDP at the same dose level. Patients who had experienced DLT were eligible for continuation of treatment with the combination after a reduction in the dose of TMZ by one dose level. No dose reduction was allowed for CDDP. CDDP was to be discontinued in patients who experienced nephrotoxicity manifested by a persistently elevated serum creatinine of >1.8 mg/dl for 2 weeks after the scheduled day of dosing. Discontinuation of CDDP in patients exhibiting severe (grade 3 or 4) neurotoxicity or ototoxicity was at the discretion of the investigator.

Drug Administration. TMZ was supplied by Schering-Plough Research Institute (Kenilworth, NJ) as white opaque, preservative-free, two-piece, hard gelatin capsules in strengths of 5, 20, 100, and 250 mg. The calculated doses of TMZ were rounded to the nearest 5 mg to accommodate capsule strength. TMZ was administered p.o. on days 1–5 to patients who fasted for at least 4 h before and 2 h after dosing. CDDP (Platinol-AQ; Bristol-Myers Squibb, Princeton, NJ) was supplied in vials of 100 mg/ml. The calculated dose of CDDP was diluted in 500 ml of normal saline containing 12.5 g of mannitol and was administered on the first day of each course, 4 h after TMZ. One liter of normal saline with 20 mEq KCl/liter, and 1 g of magnesium sulfate was administered both before and after the CDDP infusion.

The following antiemetic medications were administered prophylactically on day 1 of each course: 8 mg of ondansetron p.o. 30 min prior to TMZ, and both 32 mg of ondansetron i.v. and 10 mg of dexamethasone i.v. 30 min before CDDP. On day 2, all patients received 8 mg of ondansetron p.o. before treatment with TMZ. The prophylactic use of ondansetron before treatment with TMZ on days 3–5 was optional.

Pretreatment and Follow-Up Studies. Before each course of treatment, histories and physical examinations were performed, and the following evaluations were obtained: complete blood counts, differential WBC counts, routine chemistry and electrolyte tests, clotting studies, urinalyses, electrocardiograms, and chest radiographs. Weekly evaluations included histories, physical examinations, complete blood counts, routine chemistry and electrolyte tests, and toxicity assessments. Audiometric testing was conducted only in patients with symptoms of hearing loss or tinnitus.

Appropriate radiological studies for documentation of measurable disease were performed before enrollment and after every two courses of therapy. A complete response was scored if there was disappearance of all known disease on two measurements, separated by a minimum of 4 weeks. A partial response required at least a 50% reduction in the sum of the products of the bidimensional measurements, separated by at least 4 weeks. Progressive disease was defined as an increase in the sum of the bidimensional measurements of all known disease by at least 25% or the appearance of new lesions.

Plasma Sampling and Assay. The effect of cisplatin on the pharmacokinetic behavior of temozolomide was evaluated by sampling blood before (on day 1) and after (on day 2) cisplatin administration. Blood was collected before treatment and at 0.5, 1, 2, 3, 4, 6, and 8 h after the administration of TMZ on days 1 and 2 of course 1. The samples were collected in prechilled syringes, placed in prechilled heparinized tubes, and immediately transferred to an ice-water bath. Within 30 min of sample collection, plasma was separated by centrifugation at 3000 × g for 10 min at 4°C. After centrifugation, 2 ml of plasma were transferred to a plastic tube containing 100 µl of 8.5% phosphoric acid, vortexed briefly, and then separated into two equal portions. These specimens were frozen immediately and stored at −20°C.

Plasma samples were assayed for TMZ by high-performance liquid chromatography as described previously (34). The lower limit of quantification of TMZ was 0.1 µg/ml. Over the concentration range of 0.1–20 µg/ml, the intra- and interday precision was ≤15%. As well, the accuracy demonstrated ≤15% deviation from nominal values.

Pharmacokinetic Analysis. Individual TMZ plasma concentrations on days 1 and 2 were analyzed using model-independent methods (35). The maximum plasma concentration (Cmax) and time of maximum plasma concentration (Tmax) were determined by inspection of the plasma concentration-versus-time curves. The terminal rate constant, k (elimination phase rate constant), was calculated as the negative of the slope of the log-linear terminal portion of the plasma concentration-versus-time curve using linear regression. The terminal half-life, t1/2, was calculated as 0.693/k. The AUC from time zero to the time of the final quantifiable sample, AUC(τ), was calculated using the linear trapezoidal method and was extrapolated to infinity according to the following equation:

$$AUC_\tau = AUC(\tau) + C(\tau)/k$$

where C(τ) was the estimated concentration at time τ, CI/F (systemic clearance) was calculated by dividing the dose by AUCω, Vd/F (volume of distribution) was calculated by dividing CI/F by k.

TMZ pharmacokinetic parameters were summarized using descriptive statistics. A paired t test was used to compare pharmacokinetic parameters between days 1 and 2. Statistical analysis was performed using the JMP version 3.1.6.2 statistical software program (SAS Institute, Cary, NC).

Pharmacodynamic Analysis. The relationships between TMZ systemic exposure and toxicity were explored. Day 1 Cmax and AUCω values were used as measures of systemic exposure. Relevant parameters of myelosuppression that were evaluated included percentage decrements in the ANC and platelet count, which were calculated as:

$$\text{Percentage decrement in blood cell count} = \frac{\text{Pretreatment count} - \text{nadir count}}{\text{Pretreatment count}} \times 100$$

Both simple and sigmoidal maximum effect (Emax) models of drug effect (36) were applied to these relationships using non-
linear least-squares regression (WINNONLIN version 1.1; Statistical Consultants, Apex, NC). Linear regression models were also applied to the data. Discrimination between pharmacodynamic models was guided by minimization of the weighted sum of squares and standard errors for the pharmacokinetic parameters, examination of the dispersion of the residuals, and use of the coefficient of determination ($r^2$).

RESULTS

General. Fifteen patients, whose characteristics are detailed in Table 1, received 44 courses of TMZ and CDDP. All patients had an ECOG performance status of either 0 or 1. Six patients had received no prior therapy. Three patients had received prior CDDP, and one patient had received prior carboplatin. Three courses were not completely evaluable for toxicity because of failure of three patients to return for follow-up investigations. One patient who received one course at the 150/75 mg/m² dose level was noncompliant with follow-up laboratory studies, was taken off study, and was replaced. Two other patients, at the 150/75 and 200/75 mg/m² dose levels, were fully evaluable during their first courses but were noncompliant with follow-up laboratory studies during their second courses.

The total numbers of patients and courses as a function of TMZ/CDDP dose level and the overall dose escalation scheme are depicted in Table 2. The median number of courses received by each patient was 2 (range, 1–8). Treatment with CDDP was discontinued in a 69-year-old female after seven courses of TMZ/CDDP at the 200/75 mg/m² dose level. Though the original design of the study did not permit CDDP dose reduction, this modification enabled the patient to receive seven additional courses of TMZ/CDDP at the 200/75 mg/m² dose level.

Hematological Toxicity. Myelosuppression, particularly neutropenia, was a common toxicity of the TMZ/CDDP regimen. Table 3 displays the median values and ranges of ANC and platelet count nadirs, as well as the pertinent grades of neutropenia and thrombocytopenia, as a function of dose level. The median time to ANC nadir was 22 days (range, 8–36), with resolution of neutropenia (ANC ≥1.5) by day 28 in 30 of 41 (73%) courses. Unresolved neutropenia that resulted in treatment delays as long as 2 weeks occurred in nine courses at the following TMZ (mg/m²/day)/CDDP (mg/m²) dose levels: 150/75 (two courses), 200/75 (four courses), and 200/100 (three courses). Platelet count nadirs were also typically experienced on day 22, with recovery to pretreatment levels by day 28 in 35 of 41 (85%) courses. Thrombocytopenia (platelets <100,000/µl) contributed to treatment delays as long as 2 weeks in three courses at the 150/75 dose level.

The first two TMZ/CDDP dose levels, 100/75 and 150/75 mg/m², were well tolerated, with no evidence of grade 3 or 4 neutropenia or thrombocytopenia. At the 200/75 mg/m² dose level, 5 of 16 (31%) fully assessable courses were associated with brief (<5 days) grade 3 or 4 neutropenia. In addition, 2 of 16 (13%) courses were associated with grade 3 or 4 thrombocytopenia, and one platelet transfusion was administered. However, none of the hematological toxicities at the 200/75 dose level were dose limiting. At the highest dose level, 200/100 mg/m², 5 of 12 (42%) courses were associated with grade 3 or 4 neutropenia. One previously untreated patient with melanoma developed dose-limiting hematological toxicity characterized by grade 4 neutropenia accompanied by fever and grade 4 thrombocytopenia. She was hospitalized for treatment with parenteral antibiotics, granulocyte-colony stimulating factor, and platelet transfusions. After a dose reduction, this patient again developed grade 4 thrombocytopenia and neutropenia, for which she received granulocyte-colony stimulating factor and platelet transfusions. Neither the neutropenia nor the thrombocytopenia induced by the TMZ/CDDP regimen was cumulative, as demonstrated by Fig. 2, which depicts the mean percentage decrement in ANC and platelet count as a function of the number of administered courses.

Anemia was also a common side effect of the TMZ/CDDP

### Table 1: Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
</tr>
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<tbody>
<tr>
<td>Total number of patients</td>
<td>15</td>
</tr>
<tr>
<td>Sex (male:female)</td>
<td>7:8</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>54 (36–75)</td>
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<tr>
<td>ECOG performance status</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Previous treatment</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>6</td>
</tr>
<tr>
<td>Radiation therapy only</td>
<td>3</td>
</tr>
<tr>
<td>Chemotherapy and radiation therapy</td>
<td>3</td>
</tr>
<tr>
<td>Chemotherapy only</td>
<td>2</td>
</tr>
<tr>
<td>Biological therapy only</td>
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</tr>
<tr>
<td>Tumor type</td>
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</tr>
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<td>Non-small cell lung cancer</td>
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</tr>
<tr>
<td>Melanoma</td>
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</tr>
<tr>
<td>Primary unknown</td>
<td>3</td>
</tr>
<tr>
<td>Adenocystic carcinoma (cerumen gland)</td>
<td>1</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>1</td>
</tr>
<tr>
<td>Leiomyosarcoma (uterus)</td>
<td>1</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>1</td>
</tr>
<tr>
<td>Squamous cell carcinoma (tongue)</td>
<td>1</td>
</tr>
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</table>

### Table 2: Dose escalations

<table>
<thead>
<tr>
<th>Dose level</th>
<th>No. of patients</th>
</tr>
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<tbody>
<tr>
<td>TMZ (mg/m²/day) × 5 days</td>
<td>CDDP (mg/m²)</td>
</tr>
<tr>
<td>100 75</td>
<td>1</td>
</tr>
<tr>
<td>150 75</td>
<td>4²</td>
</tr>
<tr>
<td>200 75</td>
<td>4</td>
</tr>
<tr>
<td>200 100</td>
<td>6</td>
</tr>
<tr>
<td>150 100</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
</tr>
</tbody>
</table>

* One patient was replaced because of incomplete follow-up laboratory tests during the first course.
A total of six patients experienced symptoms indicative of ototoxicity, including two patients at the 150/75 dose level, one patient at the 200/75 dose level, and three patients at the 200/100 dose level. In three individuals, symptoms began during the first course. The ototoxicity was characterized by hearing loss (grade 3) in three patients, whereas the other three subjects experienced tinnitus (grade 2) without subjective hearing loss. Although one patient developed grade 3 ototoxicity during her first course at the 200/100 dose level, this was an anticipated toxicity of CDDP and was not considered to be dose limiting. At the discretion of the investigator, CDDP was discontinued in one patient at the 150/75 dose level who exhibited grade 3 ototoxicity during the seventh course of combination chemotherapy. This patient received one course of single-agent TMZ before disease progression.

Transient elevations in serum creatinine concentrations (grade 1 to 2) attributable to the administration of TMZ and CDDP were noted in 10 of 41 (24%) courses involving six patients across all dose levels. The serum creatinine typically increased on days 5 to 10 and returned to pretreatment levels by day 28, without clinical sequelae. In addition, grade 1 to 2 hyperbilirubinemia and hypokalemia occurred in 8 of 41 (20%) and 13 of 41 (32%) courses, respectively. These electrolyte abnormalities were readily correctable.

Two patients developed transient grade 2 to 3 hyperbilirubinemia, which was possibly related to study medication. One patient with malignant melanoma experienced isolated grade 3 hyperbilirubinemia (bilirubin, 2 mg/dl) on day 8 of course 2 at the 150/75 dose level, which resolved by day 22. This patient did not have liver metastases and was taken off study after the diagnosis of brain metastases on day 22 of course 2. A second patient with malignant melanoma experienced isolated grade 3 hyperbilirubinemia (bilirubin, 1.8 mg/dl) and concomitant grade 1 elevation of serum aspartate aminotransferase on day 8 course 3 at the 150/100 dose level. This patient’s bilirubin and transaminase returned to normal by day 10 course 3. Transient grade 1 elevations in serum transaminases were associated with 5 of 41 (12%) courses.

Other mild to moderate (grades 1 to 2) adverse effects that were possibly related to chemotherapy included fatigue in 18 of 44 (42%) courses, constipation in 14 of 44 (32%) courses, and anorexia in 13 of 44 (30%) courses. Mild to moderate peripheral neuroopathy, diarrhea, malaise, fever, and stomatitis were each noted in <20% of courses. One patient developed mild alopecia.

Antitumor Activity. Of the 14 patients who were evaluable for antitumor activity, 2 patients developed major objective responses after treatment with TMZ and CDDP. The first patient, a 69-year-old female with previously untreated non-small cell lung cancer, developed a partial response after two courses at the 150/75 dose level. The partial response was maintained for 6 months, during which time she received six additional

Table 3 Hematological toxicities of TMZ and CDDP

<table>
<thead>
<tr>
<th>Dose level* (mg/m²/day × 5 days)</th>
<th>TMZ (mg/m²)</th>
<th>CDDP (mg/m²)</th>
<th>No. of evaluable Courses</th>
<th>Neutropenia</th>
<th>Thrombocytopenia</th>
<th>Median nadir ANC/µl (range)</th>
<th>Grade 3</th>
<th>Grade 4 &gt; 5 days</th>
<th>Grade 4 + fever</th>
<th>Median nadir platelets/µl (range)</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Grade with hematological DLT/Total</th>
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<tr>
<td>100</td>
<td>75</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>5740</td>
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<td>0 0</td>
<td>255</td>
<td>0 0</td>
<td>0 0</td>
<td>0/1</td>
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</tr>
<tr>
<td>150</td>
<td>75</td>
<td>4 (3)</td>
<td>12 (11)</td>
<td>2000</td>
<td>0</td>
<td>0</td>
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<td>0 0</td>
<td>125 (69–433)</td>
<td>0 0</td>
<td>0 0</td>
<td>0/4</td>
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<tr>
<td>200</td>
<td>75</td>
<td>5 (5)</td>
<td>17 (16)</td>
<td>2555</td>
<td>3</td>
<td>2</td>
<td>0 0</td>
<td>0 0</td>
<td>110 (11–335)</td>
<td>1 1</td>
<td>1 1</td>
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</tr>
<tr>
<td>200</td>
<td>100</td>
<td>6 (6)</td>
<td>12 (12)</td>
<td>1165</td>
<td>4</td>
<td>1</td>
<td>1 1</td>
<td>1 1</td>
<td>112.5 (22–356)</td>
<td>0 1</td>
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<td>100</td>
<td>2 (1)</td>
<td>2 (1)</td>
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<td>1</td>
<td>0 0</td>
<td>0 0</td>
<td>23</td>
<td>0 1</td>
<td>0 0</td>
<td>0/2</td>
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</table>

*Oral TMZ was administered in the fasting state on days 1 through 5. CDDP was administered i.v. on the first day of each course, 4 h after the administration of TMZ. Courses were repeated every 28 days.

Fig. 2 Mean percentage decrement in ANC (○) and platelet count (○) versus the number of courses received at the TMZ (mg/m²/day)/CDDP (mg/m²) dose levels of 200/75 and 200/100. Bars, SD.
The second patient, a 54-year-old male with squamous cell carcinoma involving the tongue, cervical lymph nodes, and lungs, experienced a partial response after one course at the 200/100 dose level and one course at the reduced level of 150/100. This patient, however, declined further treatment because of the protracted grade 3 nausea and vomiting that he experienced while on study.

In addition to the two patients with partial responses, one patient developed a significant reduction in disease that was not maintained. This patient was a 62-year-old female with metastatic leiomyosarcoma of the uterus, involving the lungs, bones, and peritoneum, who had previously been treated with doxorubicin and radiation therapy to the lumbar spine. After six courses of TMZ/CDDP (one course at the 200/100 dose level and five courses at the 200/75 dose level), she developed a 61% reduction in her disease. This antitumor activity did not meet the criteria for a partial response, because it was not confirmed by a repeat radiological study. Two months later, the patient was found to have progressive disease after a total of eight courses of therapy.

Pharmacokinetic Studies. All 15 patients had complete blood sampling performed for pharmacokinetic studies of TMZ on days 1 and 2. Plasma concentration data from one patient who was treated at the 200/100 mg/m² dose level was not analyzed because undigested capsule fragments were observed in emesis on day 2. In one patient at each of the 150/75 and 200/75 mg/m² dose levels, AUC₀₋₅₀₀ C/F, and V₀₋₅₀₀/C estimates on day 2 could not be reliably determined because the extrapolated portion of the concentration-versus-time curve represented >25% of the total AUC₀₋₅₀₀(t). Representative plasma concentration-versus-time profiles on days 1 and 2 are shown in Fig. 3. The mean (± SD) TMZ pharmacokinetic parameters determined using noncompartamental methods are listed in Table 4.

The effect of CDDP on the pharmacokinetic behavior of TMZ was evaluated by determining the pharmacokinetic parameters of TMZ both before (day 1) and after (day 2) cisplatin administration. Oral TMZ was rapidly absorbed with a mean (± SD) Tₘₐₓ value of 1.1 ± 0.6 h on day 1 and a mean (± SD) Tₘₐₓ value of 1.7 ± 0.9 h on day 2 (P = 0.03). Cₘₐₓ values decreased by an average of 22% from day 1 to day 2 (P = 0.03), although AUC values were similar on days 1 and 2 (P = 0.45). There was no statistically significant difference between the TMZ clearance of 115 ± 27 ml/min/m² on day 1 and 141 ± 109 ml/min/m² on day 2 (P = 0.34). The t₁/₂, however, increased slightly but significantly from 1.74 ± 0.22 h on day 1 to 2.35 ± 0.70 h on day 2 (P = 0.005).

Pharmacodynamic Studies. Fourteen patients were evaluable for pharmacodynamic studies. The relationships between TMZ AUC₀₋₅₀₀ and the percentage decrements in either ANC or platelet count were not well described by either simple or sigmoidal Eₘₐₓ models (r² ≤ 0.12) or by linear models (r² ≤ 0.14). Cₘₐₓ was more predictive of the percentage decrements in platelet count (r² = 0.39) than ANC (r² = 0.15) using linear regression analysis (Fig. 4, A and C). The increase in myelosuppression with increasing doses of TMZ is demonstrated in Fig. 4, B and D.

DISCUSSION

The rationale for combining TMZ on a daily schedule for 5 days every 4 weeks, with CDDP on day 1, was based, in part, on the potential for improved antitumor activity compared with single-agent TMZ. Although both TMZ and CDDP target DNA, these agents have widely disparate mechanisms of cytotoxic action and induce different cytotoxic lesions. TMZ produces methyl adducts at the O₆ position of guanine via the MTIC intermediate (1–13), whereas CDDP produces DNA cross-links preferentially at the N² positions of guanine and adenine (37). The methyl adducts produced by TMZ are removed by the DNA repair enzyme AGAT (6–13), and TMZ activity correlates inversely with AGAT activity (6–9). Depletion of AGAT by the alkyltransferase inhibitors O₆-methylguanine (6, 11) and O₆-benzylguanine (7, 9, 10, 12, 13) potentiates TMZ activity in vitro and in vivo. Moreover, murine hematopoietic stem cells transfected with the human AGAT gene are protected from TMZ cytotoxicity (38), suggesting that AGAT activity confers resistance to TMZ. Conversely, the antitumor activity of single-agent CDDP is not related to AGAT activity (39).

Although AGAT does not influence the activity of CDDP, CDDP may affect AGAT activity. H4 rat hepatoma cells have an almost 4-fold increase in AGAT activity after incubation with CDDP (0.5 µM) for 1 h (40). In contrast, HeLa human cervical carcinoma cells exposed to CDDP (0–14 µM) for up to 6 days demonstrate a dose-dependent inactivation of AGAT activity (31). This inactivation may be secondary to platinated DNA adducts at the N⁷ and O₆ positions of guanine (31), although platinated RNA and protein adducts may also be implicated (32). The ability of CDDP to down-regulate AGAT is supported by the increased sensitivity of leukemic blasts to TMZ with concurrent CDDP administration (32). Simultaneous treatment of leukemic blasts with TMZ (125 µM) and noncytotoxic concentrations of CDDP (0–5 µM) produces significantly greater growth inhibition than treatment with TMZ alone (32). This preclinical evidence suggests that CDDP may enhance the antitumor activity of TMZ.

Other factors also contributed to the rationale for the combination of TMZ and CDDP. Both TMZ (21–23, 26) and CDDP (30) are active against melanoma, a tumor type with limited therapeutic options for advanced disease. In addition, TMZ and CDDP treatment may preferentially enhance the expression of AGAT (30). Finally, CDDP preferentially inhibits AGAT activity at 1–13, whereas TMZ preferentially inhibits AGAT activity at the O₆ position (6–13). A combination of these two agents may therefore act synergistically to prevent or delay the development of AGAT resistance.
CDDP have nonoverlapping DLTs, implying that the tolerable doses of TMZ and CDDP in combination are similar to the tolerable doses of single-agent TMZ and CDDP. Also, TMZ is only minimally renally excreted (4, 22, 23), suggesting that even in the presence of CDDP-induced nephropathy, CDDP does not alter the pharmacokinetic profile of TMZ. Given the potential for a favorable therapeutic index, this study explored the MTD, toxicities, and pharmacokinetic parameters of TMZ in combination with CDDP.

CDDP has previously been combined with another imidazotetrazinone, DTIC, in the treatment of metastatic melanoma. Interest in the DTIC/CDDP combination was generated after the demonstration of additive cytotoxic activity in the B16 human melanoma model (41). In one study in which 30 patients with refractory metastatic melanoma were treated with 750 mg/m² DTIC and 100 mg/m² CDDP i.v. at 21-day intervals, objective responses lasting a median duration of 31 weeks were noted (42). However, a multicenter Phase II Southwest Oncology Group study evaluating the same DTIC/CDDP regimen in patients with stage IV or inoperable stage III melanoma failed to confirm these results. In this study, significant renal and hematopoietic toxicities were observed, and a much lower response

<table>
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<th>Dose level (mg/m²/day x 5 days)</th>
<th>TMZ Cmax (ng/ml)</th>
<th>CDDP (mg/m²)</th>
<th>T_max (h)</th>
<th>t1/2 (h)</th>
<th>V_ave/F (l/m²)</th>
<th>CL/F (mL/min/m²)</th>
<th>AUC_∞ (μg · h/ml)</th>
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<tr>
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</table>

Values represent mean (± SD). C_max, maximum plasma concentration; T_max, time to maximum plasma concentration; t_1/2, elimination half-life; V_ave/F, volume of distribution; CL/F, clearance; AUC_∞, area under the plasma concentration versus time curve; NA, not applicable.

Fig. 4 Scatterplots A and C depict the relationship between day 1 TMZ C_max and percentage decrement in the ANC (A) and platelet count (C). Shown are patients who received 75 mg/m² CDDP (●) and 100 mg/m² CDDP (○). Lines, fit of a linear model to the ANC (r² = 0.15) and platelet count (r² = 0.39) data. Scatterplots B and D depict the relationship between TMZ/CDDP dose level and percent decrement in ANC (B) and platelet counts (D).
rate (14%) and a lower median survival (6.8 months) were noted (43). The differences between the two studies may have been due to the variable generation of the active agent MTIC through hepatic p450-mediated metabolism of DTIC (14). TMZ, unlike DTIC, undergoes spontaneous biochemical decomposition to MTIC at physiological pH (1–5). Thus, substituting TMZ for DTIC may result in more predictable toxicity and antitumor activity profiles.

In this study, the combination of TMZ and CDDP was well tolerated, with two patients developing DLT at the 200/100 mg/m² dose level. At this dose level, one patient experienced both grade 4 thrombocytopenia and grade 4 neutropenia with fever, requiring hospitalization. In addition, severe and persistent nausea and vomiting necessitated dose reductions in two patients at this dose level, including one patient for whom vomiting was dose limiting. Although treatment with single-agent TMZ is associated with nausea and vomiting, which is typically brief and well controlled with serotonin antagonists (21–25), the addition of 100 mg/m² CDDP resulted in more severe nausea and vomiting, which was attributed to the inherent emetogenic potential of CDDP (37). When 75 mg/m² CDDP was combined with standard doses of TMZ, no DLTs were observed. Thus, the recommended Phase II doses are 200 mg/m² TMZ daily on days 1–5 and 75 mg/m² CDDP on day 1 every 4 weeks.

The influence of CDDP on the pharmacokinetic behavior of TMZ was evaluated by sampling plasma before (day 1) and after (day 2) CDDP administration. With control of CDDP-induced emesis, TMZ absorption was expected to be constant from day 1 to day 2. Although there were modest differences in $\text{C}_{\text{max}}$ and $\text{AUC}_{\text{max}}$ between days 1 and 2, these differences were not clinically significant. Consistent with single-agent studies, TMZ was rapidly absorbed (4, 22, 24). At the recommended dose level of 200/75 mg/m², the $\text{C}_{\text{max}}$ values on days 1 and 2 were comparable with those reported previously (9.8–16 ng/ml) for 200 mg/m²/day of single-agent TMZ daily for 5 days (22, 23). Likewise, TMZ $\text{AUC}_{\text{max}}$ and clearance were unaffected by CDDP administration, with no significant change from day 1 to day 2. At the recommended dose level, the mean $\text{AUC}_{\text{max}}$ and clearance values on days 1 and 2 were within the range of mean values that have been reported previously for 200 mg/m²/day of single-agent TMZ daily for five days (AUC, 30–35 µg·h/ml, and clearance, 104–115 ml/min/m²; Refs. 22, 23). The observation that TMZ clearance was not affected by the administration of CDDP is attributed to the fact that the disposition of TMZ is principally through chemical hydrolysis, with only minimal (5–6%) excretion by renal mechanisms (4, 22, 23).

In conclusion, 75 mg/m² CDDP did not alter the tolerable dose of TMZ on a daily for 5 days schedule, nor did it substantially affect the pharmacokinetic profile of TMZ. There was minimal interindividual variability in toxicological profiles, as expected from the ability of TMZ to spontaneously form the active moiety MTIC. This potential for more predictable toxicity and more reliable antitumor activity may lead to an improved therapeutic index when TMZ is substituted for DTIC in imidazotetrazinone/CDDP regimens.

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A Phase I and Pharmacokinetic Study of Temozolomide and Cisplatin in Patients with Advanced Solid Malignancies


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