Absorption, Metabolism, and Excretion of \(^{14}\)C-Temozolomide following Oral Administration to Patients with Advanced Cancer\(^1\)

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ABSTRACT

The purpose of this study is to characterize the absorption, metabolism, and excretion of carbon \(^{14}\)labeled temozolomide (\(^{14}\)C-TMZ) administered p.o. to adult patients with advanced solid malignancies.

On day 1 of cycle 1, six patients received a single oral 200-mg dose of \(^{14}\)C-TMZ (70.2 \(\mu\)Ci). Whole blood, plasma, urine, and feces were collected from days 1–8 and on day 14 of cycle 1. Total radioactivity was measured in all samples. TMZ, 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC), and 4-amino-5-imidazole-carboxamide (AIC) concentrations were determined in plasma, and urine and plasma samples were profiled for metabolite/degradation products.

Maximum TMZ plasma concentrations were achieved between 0.33 to 2 h (mean, 1.2 h), and half-life, apparent volume of distribution, and oral clearance values averaged 1.9 h, 17 liters/m\(^2\), and 104 ml/min/m\(^2\), respectively. First-order absorption, one-compartment linear model, which included first-order formation of MTIC from TMZ and elimination of MTIC via degradation to AIC, and a peripheral distribution compartment for AIC, adequately described the plasma TMZ, MTIC, and AIC concentrations. MTIC systemic clearance was estimated to be 5384 ml/min/m\(^2\), and the half-life was calculated to be 2.5 min. Metabolite profiles of plasma at 1 and 4 h after treatment showed that \(^{14}\)C-derived radioactivity was primarily associated with TMZ, and a smaller amount was attributed to AIC. Profiles of urine samples from 0–24 h revealed that \(^{14}\)C-TMZ-derived urinary radioactivity was primarily associated with unchanged drug (5.6%), AIC (12%), or 3-methyl-2,3-dihydro-4-oxoimidazo[5,1-d]tetrazine-8-carboxylic acid (2.3%). The recovered radioactive dose (39%) was principally eliminated in the urine (38%), and a small amount (0.8%) was excreted in the feces.

TMZ exhibits rapid oral absorption and high systemic availability. The primary elimination pathway for TMZ is by pH-dependent degradation to MTIC and further degradation to AIC. Incomplete recovery of radioactivity may be explained by the incorporation of AIC into nucleic acids.

INTRODUCTION

TMZ\(^3\) is an alkylating agent of the imidazotetrazine series that has demonstrated notable antitumor activity in patients with recurrent and refractory high-grade glioma and melanoma in phase I and II trials (1–6). The results of both preclinical and phase I studies indicate that TMZ is completely bioavailable (F \(\sim\) 100%) when administered p.o. (1) and that antitumor activity is schedule-dependent (7, 8). Prominent clinical activity was observed principally on frequent dosing schedules, particularly when TMZ is given p.o. once a day for 5 days every 4 weeks (1–6). Present clinical developmental efforts are defining the role for TMZ in treating patients with high-grade glioma, melanoma, and other various malignancies.

It has been proposed that TMZ and DTIC, a structurally related alkylating agent that is used clinically to treat melanoma and lymphoma, exert their antitumor activity by generating the linear triazene MTIC (7, 9), which reportedly alkylates the \(O^6\)-position of guanine in DNA, with additional alkylation also occurring at the \(N^7\)-position (9–12). Unlike DTIC, which re-

\(^3\) The abbreviations used are: TMZ, temozolomide; MTIC, 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide; AIC, and 4-amino-5-imidazole-carboxamide; TMA, 3-methyl-2,3-dihydro-4-oxoimidazo[5,1-d]tetrazine-8-carboxylic acid, temozolomide acid; \(^{14}\)C-TMZ, carbon \(^{14}\)labeled temozolomide; DTIC, dacarbazine; HPLC, high performance liquid chromatography; IS, internal standard; LOQ, lower limit of quantitation; \(C_{\text{max}}\), maximum concentration; \(T_{\text{max}}\), time of maximum concentration; \(\text{AUC}\), area under the plasma or blood concentration-time curve; \(\text{Cl}_d,F\), temozolomide systemic clearance; \(V_{\text{m注射}}/F\), temozolomide apparent volume of distribution, \(T_{\text{lag}}\), lag time; \(k_f\), first-order absorption rate constant; \(V_{\text{d}},F\), volume of distribution for TMZ and MTIC, and the central volume of distribution for AIC; \(V_{\text{p},\text{AIC}}/F\), peripheral volume of distribution for AIC; \(\text{Cl}_{\text{MTIC}}/F\), clearance for the conversion of MTIC to AIC; \(\text{Cl}_{\text{AIC}}/F\), AIC distribution clearance; \(\text{Cl}_{\text{AIC}}/F\), AIC systemic clearance; elimination rate constant (\(k_e\)); CV, coefficient of variation, tf, time of final quantifiable sample; \(\text{AUC}(0–24\ h)\), AUC from time 0–24 h; \(\text{AUC}(0)\), AUC from time zero to infinity; \(t_{1/2}\), terminal half-life.
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Increased proportionally with dose, and no accumulation of drug of 1.8 h. Maximum plasma concentrations and AUC values peak plasma concentration of 1 h and a mean terminal half-life TMZ was rapidly absorbed and eliminated, with a mean time to concentrations of TMZ and TMA were determined in urine. TMZ, MTIC, AIC, and TMA were determined in plasma, and its degradation products were characterized in patients with solid malignancies treated with TMZ ranging from 50 –250 mg/m²/day given daily for 5 days (5, 13, 16). Concentrations of TMZ and TMA represented approximately 2.2%, 40%, and 0.8% of systemic exposure to TMZ, respectively. The mean urinary excretion of TMZ and TMA was minimal with values of 5.6% and 0.8%, respectively.

Although the antitumor activities of TMZ in patients with melanoma and high-grade glioma have been well characterized and pivotal clinical efficacy evaluations are in progress, a comprehensive characterization of the absorption and systemic availability, metabolism/degradation and disposition in blood and plasma and total body excretion of TMZ and its degradation products after oral administration has not been performed. The principal objective of this study was to define the absorption, metabolism, and excretion of oral TMZ after administration of a single dose of 14C-TMZ to patients with advanced cancer. A secondary goal was to develop a pharmacokinetic model to: (a) describe the plasma disposition of TMZ and its degradation/bioconversion products MTIC and AIC; and (b) characterize the kinetics of the conversion of TMZ to MTIC and the subsequent degradation of MTIC to AIC in these patients.

**Fig. 1** Chemical structure of TMZ and proposed metabolism and degradation pathways. +, the position of the 14C-labeled carbon atom.

**PATIENTS AND METHODS**

**Patient Selection.** Male or female patients with histologically confirmed advanced solid malignancies who failed to respond to standard therapy or for whom adequate therapy was not available were eligible for this study. Eligibility criteria also included: age ≥18 years; an Eastern Cooperative Oncology Group Performance Status ≤ 2 (ambulatory and capable of self-care); a life expectancy of ≥12 weeks; adequate hematopoietic (absolute neutrophil count ≥1,500/µl, platelet count ≥100,000/µl, hemoglobin ≥10.0 g/dl), hepatic (total bilirubin within the upper limits of institutional laboratory normal, and aspartate aminotransferase or alanine aminotransferase and alkaline phosphatase ≤2 times the upper limit of institutional laboratory normal), and renal (serum creatinine within the upper limits of institutional laboratory normal) functions; no history or evidence of a medical condition that might affect gastrointestinal function; no surgical resection of the stomach or small bowel; no chemotherapy or biological therapy within 4 weeks of treatment (6 weeks if the previous chemotherapy regimen included mitomycin C or a nitrosourea); no radiation therapy to >50% of bone marrow; and no other coexisting medical conditions of sufficient severity to prevent full compliance with the study. Patients gave informed, written consent according to federal and institutional guidelines.

**Study Design.** This single institution phase I study was designed to characterize the absorption, metabolism, and excretion of 14C-TMZ administered p.o. to six adult patients with advanced cancer. On day 1 of cycle 1, patients received a single dose of 14C-TMZ. Blood, urine, and feces samples were collected from days 1–8 and on day 14 of cycle 1 for pharmacokinetic studies. Patients were hospitalized for the intensive pharmacokinetic evaluation on days 1–8, after which they received their remainder of their TMZ (unlabeled) treatment on days 8 –11 of cycle 1 at a dose of 150 mg/m²/day for 4 consecutive days. After completion of cycle 1, patients were treated with TMZ (150 mg/m²/day) for 5 consecutive days every 28 days, with dose adjustments made according to hematological tolerance. Treatment was continued in this phase of the study, which is not summarized in this study, as long as disease progression did not occur.
**Drug Dosage and Administration.** Patients were administered a 200-mg dose of TMZ containing 70.2 μCi of \(^{14}\)C-TMZ with 8 ounces of noncarbonated water in the morning after an overnight fast. Patients continued to fast until 4 h after treatment. To ensure uniform hydration and adequate urine collection, patients were instructed to drink 8 ounces of noncarbonated water at 2, 6, 10, and 14 h after treatment. Approximately 1 h before administration of \(^{14}\)C-TMZ, patients were treated with i.v. ondansetron (32 mg) to prevent nausea and vomiting. Any medication that could potentially influence gastric pH or drug absorption (e.g., histamine-2 antagonists, omeprazole, sulcrafate) were withheld for a period of 48 h before treatment and 8 h after treatment. Patients were not treated with antacids for a period of 12 h before TMZ treatment and 8 h after treatment. Radiolabeled TMZ was supplied as capsules of 100 mg of \(^{14}\)C-TMZ (35.1 μCi; specific activity ∼0.375 μCi/mg) by Schering-Plough Research Institute (Kenilworth, NJ). The \(^{14}\)C label was on the sixth position of the imidazole ring of TMZ, as shown in Fig. 1.

**Sample Collection and Processing.** Blood samples were collected in prechilled heparinized tubes and immediately placed in an ice-water bath. Two blood samples were collected before treatment, at 20 and 40 min, and at 1, 1.5, 2, 3, 4, 8, 12, and 24 h after \(^{14}\)C-TMZ administration. The first blood sample (∼1.5 ml) was drawn for MTIC plasma concentration determinations. Plasma was immediately separated by centrifugation (4°C), divided into two aliquots, frozen immediately in a dry ice-chromal bath, and stored at −80°C, pending analysis of MTIC. The second blood sample (∼7.5 ml) was collected for blood and plasma radioactivity and TMZ and AIC plasma concentration determinations. Before centrifugation, 2 × 0.2-mL aliquots of whole blood were transferred to two combustion vials, and immediately frozen at −20°C. Within 20 min after collection, the remaining blood was centrifuged (4°C), plasma was placed in a plastic tube containing 0.2 ml of 8.5% phosphoric acid to chemically stabilize TMZ, mixed, the pH adjusted to pH 4 (with additional phosphoric acid, if necessary), and then frozen at −80°C. Blood samples were also collected at 48, 72, 96, 120, 144, and 168 h after \(^{14}\)C-TMZ and AIC plasma concentration determinations. Before centrifugation, 2 × 0.2-mL aliquots of whole blood were transferred to two combustion vials fitted with a combustion pad, placed into scintillation vials, and immediately frozen at −20°C. Within 20 min after collection, the remaining blood was centrifuged (4°C), plasma was placed in plastic tubes containing 0.2 ml of 8.5% phosphoric acid, acidified with 70% peracetic acid, if necessary, and stored at −80°C, pending analysis of MTIC. The second blood sample (∼7.5 ml) was collected for blood and plasma radioactivity and TMZ and AIC plasma concentration determinations. Before centrifugation, 2 × 0.2-mL aliquots of whole blood were transferred to two combustion vials, and immediately frozen at −20°C. Within 20 min after collection, the remaining blood was centrifuged (4°C), plasma was placed in a plastic tube containing 0.2 ml of 8.5% phosphoric acid to chemically stabilize TMZ, mixed, the pH adjusted to pH 4 (with additional phosphoric acid, if necessary), and then frozen at −80°C. Blood samples were also collected at 48, 72, 96, 120, 144, and 168 h after \(^{14}\)C-TMZ administration and similarly processed for determination of blood and plasma radioactivity and TMZ and AIC concentrations in plasma.

Additional blood samples (∼22 ml) were collected at 1 and 4 h after \(^{14}\)C-TMZ administration to assess \(ex vivo\) protein binding and for metabolite profiling. Blood samples were centrifuged (4°C) and 3 × 1-ml aliquots of plasma were transferred to the sample reservoir of three separate ultrafiltration devices. The ultrafiltration devices were centrifuged at 1000–2000 g for 20 min at 37°C, and the filtrate cup was removed and frozen at −20°C. The remaining plasma was divided into two equal portions, acidified with 0.2 ml of 8.5% phosphoric acid, adjusted to pH 4, if necessary, and frozen at −20°C until assayed for a metabolite profile(s).

Urine was collected in plastic containers containing 2 ml of 8.5% phosphoric acid solution at baseline and then continuously during the following timed collections: 0–4, 4–8, 8–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 h after \(^{14}\)C-TMZ administration. An additional 24-h urine collection was obtained between days 13 and 14. After each void, the pH was determined and adjusted to pH 4 with additional 8.5% phosphoric acid, if necessary. For the first 48 h, the entire volume of urine collected during each interval was retained; for all other intervals, the total volume of urine was recorded at the end of the collection period, and 2 × 50-ml aliquots were frozen at −20°C until assayed for radioactivity.

A fecal sample was collected before drug administration, and then all feces were collected continuously until 168 h after administration of \(^{14}\)C-TMZ. An additional fecal sample was collected on day 14. The fecal samples were transferred to plastic containers and immediately frozen at −20°C until assayed for radioactivity.

**Sample Preparation and Analysis.** All samples were assayed for total radioactivity using liquid scintillation spectrometry with external standardization methods. Plasma samples also were assayed for TMZ, MTIC, and AIC using HPLC and UV detection with modifications of previously described procedures (13, 14, 17). Briefly, plasma samples were enriched with ethazolastone solution (IS) and extracted with ethyl acetate. Samples were centrifuged, and the organic layer was removed and completely evaporated under a stream of nitrogen. The final extract was reconstituted with HPLC mobile phase [0.1% acetic acid and acetonitrile (90/10, v/v)] and analyzed by reversed-phase HPLC. Samples were injected onto an Ultraphase octadecyl silane column equipped with a silica guard column, and column eluate was monitored at 316 nm. Over the range of 0.100–20.0 µg/ml, the TMZ assay precision (percentage of CV) was 6.7%, accuracy (percentage of bias) was 2.8%, and the LOQ in plasma was 0.100 µg/ml.

For MTIC determinations, plasma samples were enriched with ice-cold metronidazole solution (IS), and plasma proteins were precipitated with isopropyl alcohol. Samples were vortex mixed, centrifuged (4°C), and a portion of the resultant supernatant was diluted in mobile phase (50 mM ammonium acetate containing 2% isopropyl alcohol). Extracts were analyzed using a C8 reversed-phase column protected by a C18 guard column, and MTIC was detected at 316 nm. Over the range of 25–1000 ng/ml, assay precision and accuracy was 14% and 5.1%, respectively, and the LOQ for MTIC in plasma was 25 ng/ml. To determine AIC plasma concentrations, samples were enriched with AICO solution (IS) and extracted by solid-phase extraction using a strong cation-exchange column. AIC was eluted from the cation-exchange resin using methanol containing 0.6% ammonium hydroxide. The resulting eluate was dried under a stream of nitrogen, and reconstructed with methanol. The mobile phase consisted of 0.1% trifluoroacetic acid (adjusted to pH 2.5 with triethylamine) and methanol. Separations were performed on an octadecyl silane reversed-phase column using a gradient elution system, and AIC was detected at 265 nm. AIC assay precision (10%) and accuracy (4.5%) were acceptable over the concentration range of 20–2000 ng/ml, and the LOQ for AIC in plasma was 20 ng/ml.

Plasma samples, collected at 1 and 4 h after treatment for metabolite profiles, were pooled from all six patients at each time point, and ∼1% of the total urine volume excreted from each patient within each of the 0–4, 4–8, 8–24, 24–48, and 48–72-h collection intervals was pooled for determination of metabolite profiles. Pooled plasma samples were extracted by protein precipitation using methanol acidified with 70% per-
chboroic acid, mixed, and centrifuged. This and all subsequent centrifugation steps were performed at ambient temperature. The supernatant was filtered (0.45 μm) and transferred into a glass tube, reduced in volume to 250–500 μl under a stream of nitrogen (30°C), and centrifuged. The clarified supernatant was transferred to a microcentrifuge tube equipped with a filter (0.2 μm), centrifugated at 15,800 × g for 5 min, and hexane sulfonic acid was added to achieve a 50-mM concentration. A standard solution containing 500 μg/ml each of TMZ, TMA, and AIC was prepared in 50 mM hexane sulfonic acid in 0.5% acetic acid, and a 30-μl aliquot of this standard solution was added to each sample and mixed before chromatographic analysis.

Pooled urine from each collection time interval was freeze-dried for ~16 h (lyophilized volume: ≤8.4 ml for the 0–4-, 4–8-, and 8–24-h collection interval; ≥20 ml for the 24–48- and 48–72-h collection period). Lyophilized residues from the 0–4-, 4–8-, and 8–24-h samples were each reconstituted into 100 μl of the standard solution along with an additional 100 μl of 50 mM hexane sulfonic acid in 0.5% acetic acid, and then centrifuged at 2000 × g for 5 min. Twenty (20) and 150-μl aliquots of the clarified supernatant were used to determine total radioactivity and profile metabolites, respectively. Due to the high endogenous UV background that was observed with smaller volumes of lyophilized urine, the 24–48- and 48–72-h samples were expected to saturate detector response at 254 nm. Therefore, lyophilized residues from these collection periods were not enriched with standard mixture, but were instead reconstituted into 500 μl of 50 mM hexane sulfonic acid in 0.5% acetic acid and processed in the same manner as those from the earlier collection time points. Plasma and urine samples were not evaluated for the presence of conjugated metabolites because previous studies had shown that TMZ and/or its metabolites are unstable under conditions normally used for enzymatic hydrolysis (data not shown).

Chromatographic analysis for metabolite profiling was performed using an HPLC system consisting of a Waters 600E System Controller, a Waters Model 717 plus Autosampler, a Supelco LC-DB C18 (4.6 × 250 mm) HPLC column, a Waters 18 Symmetry guard column, and a Waters Model 486 Tunable Absorbance Detector at 254 nm. In-line detection of radioactive effluent was performed on a Packard Instruments Radioumatic A250 Flo-One radiometric flow detector. Packard Ultima-Flo liquid scintillation mixture was mixed post-column with the eluent at a 3:1 ratio (v/v). For plasma samples that contained concentrations of radioactivity too low for in-line detection, 0.5-min fractions were collected postradioactive detector using an ISCO Foxy fraction collector; fractions were then mixed with 5 ml of Ultima Gold XR liquid scintillation fluid, which in turn were analyzed using a Packard Tri-Carb Model 2550 TR/LL Liquid Scintillation Analyzer. A linear gradient elution program using 50 ml hexane sulfonic acid prepared in methanol and 50 ml hexane sulfonic acid in 0.5% acetic acid was generated by the Waters 600E System Controller set at Gradient #6. Separations were accomplished at 31°C using an Eppendorf CH-30 Column Heater; and the mobile phase flow rate was maintained at 1 ml/min. The signal outputs from the UV and radiometric detectors were collected using the Flo-One software.

Additionally, plasma samples obtained at 1 and 4 h were assayed by an ultrafiltration procedure to determine the extent of protein binding. Three 1-ml aliquots of plasma were centrifuged (37°C) at 1000–2000 × g for 20 min using Centrifree micropartition tubes (Amicon). Because of the known instability of TMZ in plasma at physiological pH (7–9, 14), protein binding due to total drug-derived radioactivity was evaluated rather than that of TMZ. Unfiltered plasma and ultrafiltrate radioactivity concentrations were used to determine the percentage of protein-bound radioactivity and the free fraction of radioactivity. Unfiltered radioactivity concentrations were adjusted by a dilution factor, which corrected for the addition of phosphoric acid to the plasma samples. The percentage of protein-bound radioactivity was calculated by subtracting the ultrafiltrate concentration from the calculated unfiltered concentration, dividing the result by the calculated unfiltered concentration, and multiplying the results by 100. The free fraction was calculated by subtracting the percentage bound from 100.

Pharmacokinetic Analysis. Individual concentrations above the individual matrix LOQs for radioactivity, TMZ, MTIC, and AIC in plasma and radioactivity in blood were analyzed using model-independent methods (18). The Cmax and Tmax were the observed values on inspection of the concentration-time curves. The terminal rate constant, k, was calculated as the negative of the slope of the log-linear terminal portion of the plasma concentration-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(tf), was calculated using the linear trapezoidal method and was extrapolated to infinity (1) according to the following equation:

\[
\text{AUC(I)} = \text{AUC(tf)} + \frac{C(tf)}{K}
\]

where C(tf) was the estimated concentration at time tf. When the concentration of TMZ, MTIC, or AIC at 24 h was below the LOQ, the k was determinable, AUC(I) was used to approximate the AUC from time 0–24 h, AUC(0–24 h).

Otherwise, AUC(0–24 h) was also calculated using the linear trapezoidal method. TMZ Cls/F was calculated as the dose divided by AUC(I). TMZ Vd/F was calculated by dividing Cl/F by k. Relative systemic exposure of MTIC and AIC to that of TMZ was calculated as the AUC(0–24 h) ratio of MTIC/TMZ and AIC/TMZ, respectively.

Plasma concentration data for TMZ, MTIC, and AIC were also analyzed using model-dependent methods. A first-order absorption, one-compartment linear model, which included first-order metabolite formation and elimination and a peripheral distribution compartment for AIC (Fig. 2), was used to simultaneously describe TMZ, MTIC, and AIC plasma disposition. The following assumptions were included: (a) the absorbed drug was in the form of TMZ, where TMZ is assumed to be stable at the acidic pH of the stomach and small bowel; (b) clearance of TMZ from plasma was due to pH-dependent, chemical conversion of parent compound to MTIC and subsequent breakdown of MTIC to AIC (7–9, 13, 14), although a small percentage of TMZ is excreted unchanged in the urine (5–10%; Ref. 5) and metabolized to TMA (1–2%; Refs. 5 and 15); (c) because TMZ degrades in all tissues by pH-dependent hydrolysis, MTIC and AIC are formed within the same volume as that for TMZ, and MTIC has the same volume of distribution as TMZ; and (d) AIC has an additional distribution compart-
ment that is consistent with the partitioning of AIC into erythrocytes, where AIC is known to undergo phosphorylation (19).

The compartmental model consisted of the following eight structural parameters: $T_{lag}$, $k_v$, $V/F$, $V_{p,AIC}/F$, $Cl_s,MTIC/F$, $Cl_s,AIC/F$, and $Cl_p,AIC/F$. Individual pharmacokinetic parameters were estimated by the method of weighted least-squares regression as implemented in Adapt II release 3 (20). Initially, values for $T_{lag}$, $k_v$, $V/F$, and $Cl_s,MTIC/F$ were estimated by fitting a first-order absorption, one-compartment model to TMZ concentrations. These values were then fixed for each patient, and the complete model was fit to MTIC and AIC concentrations to estimate values for $V_{p,AIC}/F$, $Cl_s,MTIC/F$, $Cl_s,AIC/F$, and $Cl_p,AIC/F$. The elimination rate constant, $k_e$, was calculated for both TMZ and MTIC by dividing $Cl/F$ (for each moiety) by $V/F$, and the elimination $t_{1/2}$ was calculated as $0.693/k_e$. Systemic exposure for MTIC and AIC to that of TMZ was calculated as the ratio of $Cl_s,MTIC/F:Cl_s,AIC/F$, and $Cl_s,TMZ/F:Cl_s,AIC/F$, respectively (21).

Urinary excretion of TMZ, TMA, and AIC, expressed as a percentage of dose, were calculated for each collection interval by dividing the amount of excreted radioactivity that coeluted with unlabeled standard (TMZ, TMA, and AIC) by the total administered radioactive TMZ dose. These calculations were based on the following assumptions: (a) the recovery of all radioactivity was constant throughout sample processing (e.g., there was no disproportionate loss of any single metabolite); and (b) the absolute response of the radiometric detector did not change throughout HPLC analysis using gradient elution. In addition, all drug-related radioactivity in urine after 24 h was assumed to be associated with an unidentified polar metabolite(s).

RESULTS

Six males with the following tumor types received a single dose of 200 mg of $^{14}$C-TMZ (70.2 μCi): melanoma (four patients), colorectal (one patient), and pyriform sinus (one patient). The median age was 56 years (range, 32–77); the median BSA was 1.92 (range, 1.52–2.37). All patients completed the pharmacokinetic evaluations.

Pharmacokinetics of Radioactivity, TMZ, MTIC, and AIC. Mean plasma and blood radioactivity concentration-time profiles are illustrated in Fig. 3, and mean pharmacokinetic parameters are summarized in Table 1. Blood and plasma radioactivity concentrations were detectable at the first sampling time point (0.33 h) and remained quantifiable at 168 h. Mean blood and plasma $C_{max}$ values were 4.7 and 5.8 μg/ml, respectively. The ratio of blood to plasma radioactivity concentrations increased after 12 h after administration of post-$^{14}$C-TMZ, which is reflected in a higher mean AUC(t) value for radioactivity in blood (137 μg*hr/ml) than in plasma (83 μg*hr/ml). Mean TMZ pharmacokinetic parameters determined using noncompartmental methods are shown in Table 1. TMZ was rapidly absorbed; maximum plasma concentrations were observed from 0.33–2.0 h (mean, 1.2 h). TMZ $C_{max}$ value averaged 5.2 μg/ml, which represented 90% of the total radioactivity in plasma. TMZ was rapidly eliminated from plasma with concentrations not quantifiable after 8 h after treatment in five of six patients. Mean $t_{1/2}$ and $V_{area}/F$ values were 1.9 h and 17 liters/m$^2$, respectively. The TMZ oral clearance averaged 104 ml/min/m$^2$, and interindividual variability, expressed as CV, was 20%. On the basis of the ratio of plasma radioactivity to the plasma TMZ AUC(0–24 h), TMZ accounted for ~45% of the plasma radioactivity during the 24-h period after treatment.

MTIC and AIC pharmacokinetic parameters that were derived from the noncompartmental analysis are summarized in Table 1. Similar to TMZ, the mean $T_{max}$ value for MTIC was 1.5 h. Maximum AIC plasma concentrations were achieved later than TMZ and MTIC with a mean $T_{max}$ value of 2.5 h (range, 1–4 h). In the majority of patients, MTIC and AIC concentrations were not quantifiable beyond 8 and 12 h after treatment, respectively, and apparent $t_{1/2}$ values averaged 2.1 and 2.6 h, respectively. MTIC $t_{1/2}$ values could not be calculated for two patients due to the lack of a definitive elimination phase and large errors in the estimation of the terminal rate constant (k) by linear regression. Mean systemic exposure to MTIC was 2.4% (range, 1.5–4.2) that of TMZ. Mean systemic exposure to AIC was ~23% (range, 17–26) that of TMZ. The combined mean AUC(0–24 h) values for TMZ, MTIC, and AIC represented ~56% of the (0–24 h) plasma radioactivity.

![Fig. 2](image-url) Compartmental model for TMZ, MTIC, and AIC concentrations in plasma.

![Fig. 3](image-url) Mean blood (△) and plasma (○) radioactivity concentrations after single oral administration of 200 mg of $^{14}$C-TMZ. Bars, the SD.
Plasma concentrations of TMZ, MTIC, and AIC were well described by the compartmental model shown in Fig. 2. Concentration-time profiles of model-predicted and observed TMZ, MTIC, and AIC concentrations from a representative patient are illustrated in Fig. 4. In all patients, the appearance and elimination of MTIC from plasma paralleled that for TMZ. Model-estimated pharmacokinetic parameters are presented in Table 2. Results from the compartmental analysis are in good agreement with those obtained using noncompartmental methods; mean values for TMZ volume of distribution, half-life, and clearance, and systemic exposure to MTIC and AIC were within 5% of each other. MTIC systemic clearance was high (mean, 5384 ml/min/m²), with a corresponding mean t1/2 value of 2.5 min.

AIC disposition was characterized by mean values for Vp,AIC, Cls,AIC/F, and Cld,AIC of 35 liters/m², 410 ml/min/m², and 1908 ml/min/m², respectively.

Excretion of Radioactivity. Mean cumulative recovery, including the additional 24-h urine and feces collection on day 14 was 39% (range, 3245). On average, <1% of the 14C-TMZ dose was recovered in the feces and 38% was recovered in the urine over the 360-h collection period. Approximately 68% of radioactivity recovered in the urine was excreted within 24 h after treatment in all patients. A small amount (mean, 0.7%) of radioactivity was recovered in urine on day 14.

Metabolite Profiles. The metabolite profiles from plasma pooled at 1 and 4 h are shown in Fig. 5. Mean recovery of radioactivity from plasma after extraction was 64%. At 1 h (Fig. 5A), the majority of the radioactivity was associated with retention times consistent with TMZ (74%) and AIC (9.4%) standards. Radioactivity (13%) observed before the void volume is believed to be trace contamination from a previous run with radiolabeled standards. In the 4-h pooled plasma sample (Fig. 5B), a significant amount (89%) of radioactivity was observed in the region coincident with parent drug (RT; 12.5 min), whereas AIC (RT ~ 27 min) only accounted for 4.8% of total radioactivity detected in the chromatogram. A minor amount (4.7%) of radioactivity was associated with unidentified polar metabolites eluting between 4.5 and 7.0 min.

The urinary metabolite profiles are shown in Fig. 6. Mean recovery of urinary radioactivity after lyophilization was 87%.

| Table 1 | Mean pharmacokinetic parameters: noncompartmental analysis of radioactivity in blood and plasma and TMZ, MTIC, and AIC concentrations in plasma |
| Parameter | Unit | Blood radioactivity | Plasma radioactivity | TMZ | Unit | MTIC | AIC |
| Cmax | µg/ml | 4.7 (24) | 5.8 (25) | 5.2 (34) | ng/ml | 133 (37) | 657 (24) |
| Tmax | hr | 1.6 (72) | 1.3 (74) | 1.2 (59) | hr | 1.5 (62) | 2.5 (49) |
| AUC (t) | µg·hr/ml | 137 (9) | 83 (16) | 16 (13) | ng·hr/ml | 628 (113) | 3591 (8) |
| tf | hr | 268 (0) | 152 (26) | 8.7 (19) | hr | 8.3 (96) | 12 (0) |
| AUC (0–24 hr) | µg·hr/ml | 38 (15) | 38 (14) | 17 (12) | ng·hr/ml | 410 (53) | 3824 (10) |
| AUC (I) | µg·hr/ml | NC | NC | 17 (12) | ng·hr/ml | 410 (53) | 3824 (10) |
| t1/2 | hr | NC | NC | 1.9 (4) | hr | 2.1 (52) | 2.6 (16) |
| Cls,F | ml/min/m² | NC | NC | 104 (20) | ml/min/m² | NC | NC |
| Vp,F | liter/m² | NC | NC | 17 (22) | liter/m² | NC | NC |
| AUC(I) ratio of MTIC:TMZ | | | | | 0.024 (59) |
| AUC(I) ratio of AIC:TMZ | | | | | 0.23 (15) |

\[ ^a \text{µg, µg}^\text{eq for radioactivity.} \]

\[ ^b \text{NC, not calculated.} \]

| Table 2 | Mean pharmacokinetic parameters: compartmental analysis for TEM, MTIC, and AIC in plasma |
| Parameter | Unit | Mean | % CV |
| Tlag | hr | 0.52 | 28 |
| ka | hr⁻¹ | 3.0 | 95 |
| Vp,F | liter/m² | 17 | 25 |
| Vp,AIC/F | liter/m² | 35 | 51 |
| Cls,TMZ/F | ml/min/m² | 108 | 21 |
| Cls,MTIC/F | ml/min/m² | 5384 | 58 |
| Cls,AIC/F | ml/min/m² | 410 | 44 |
| Cls,AIC/F | ml/min/m² | 1908 | 120 |
| Cls,MTIC/F | ml/min/m² | 21 |
| Cls,AIC/F | ml/min/m² | 28 |
| Cls,AIC/F | ml/min/m² | 16 |

\[ ^a \text{µg, µg}^\text{eq for radioactivity.} \]

\[ ^b \text{NC, not calculated.} \]
The majority of radioactivity in the 0–4-h urine sample (Fig. 6A) was associated with retention times consistent with TMZ (44%), AIC (39%), and TMA (6.9%) standards. Minor highly polar peaks (four peaks representing a combined 10.3%) were observed early (8.9 min) in the chromatogram. The majority of radioactivity in the 4–8-h sample (Fig. 6B) was associated with AIC (55%), followed by TMZ (21%) and TMA (7.1%) standards. There was a slight increase in the relative contribution from the four unknown polar metabolites (13% detected between 2 and 9.1 min). The composite urinary profile at 8–24 h after treatment showed no radioactivity coeluting with TMZ (data not shown); decreasing amounts of radioactivity associated with both TMA and AIC coeluted standard were observed along with increasing amounts of the very polar metabolite(s) eluting early in the chromatogram. By 24–48 h, the majority of the radioactivity was associated with polar metabolites, which were poorly retained ($R_t < 6$ min). No reversed-phase HPLC profiling was performed on the residue after lyophilization of the 48–72-h pooled urine sample, due to poor solubility of the final extract and low overall recovery (~50%) of radioactivity. Urinary metabolites expressed as percentage of dose are shown in Table 3. $^{14}$C-TMZ-derived radioactivity (percentage of dose) was eliminated in urine as unchanged drug (5.5%), AIC (11.9%), TMA (2.3%), and unidentified polar compounds (17%).

### Plasma Protein Binding

Plasma protein binding at 1 and 4 h showed mean free fractions of radioactivity of 84% (range, 64–92) and 88% (range, 75–92), respectively. An average of 12–16% of drug-derived radioactivity was bound to plasma proteins.

### DISCUSSION

TMZ, an imidazotetrazine derivative, is a new oral alkylating agent that has demonstrated promising antitumor activity in phase I and II trials (1–6), particularly in patients with advanced high-grade central nervous system malignancies and...
melanoma. Mechanistic and preclinical studies predicted a potential clinical advantage of TMZ based, in part, on the nonenzymatic, pH-dependent generation of the cytotoxic intermediate MTIC, which optimally occurs at physiological pH (7–9). This clinical study provides evidence that the principal pathway for TMZ elimination is through the formation of MTIC. The observed low interpatient variability in TMZ clearance indicates that generation of MTIC is consistent between patients. These results, combined with the observed high systemic availability after oral administration, indicate that the clinical use of TMZ may lead to a significant therapeutic advantage over i.v. DTIC, which requires metabolic activation via cytochrome P450 enzymes.

In this 14C-pharmacokinetic study, TMZ exhibited rapid absorption and high systemic availability after single oral administration of 200 mg of 14C-TMZ. Mean maximum TMZ plasma concentrations were achieved at 1.2 h, which represented the majority (90%) of radioactivity in plasma (Table 1). On the basis of the observed negligible fecal excretion of radioactivity (<1% of dose), >99% of the oral TMZ dose was absorbed. These results are consistent with those reported in previous phase I studies, in which time to peak plasma concentrations were reported to be 1.0 h (5), and bioavailability after oral administration of an earlier capsule formulation to five patients was ~100% (1).

After the absorption of 14C-TMZ, the systemic exposure to radioactivity, TMZ, and the degradation products MTIC and AIC were characterized. The ratio of blood:plasma radioactivity concentrations increased over time between 12 and 168 h (Fig. 3), which may have been due to the partitioning of AIC into erythrocytes, where AIC is known to undergo phosphorylation (19). To support this, AIC was formed and quantitated in all patients. AIC concentrations were determined in plasma up to 12 h after treatment; AIC exposure represented ~23% of systemic exposure to TMZ (Table 1). Furthermore, metabolite profiling of radioactivity in plasma at 1 and 4 h after 14C-TMZ administration revealed that 4.8–9.4% of the radioactivity was associated with AIC (Fig. 5). MTIC also was measurable in all patients and represented ~2.4% of systemic exposure to parent compound (Table 1). Metabolite profiling showed that TMZ accounted for 74% and 89% of plasma radioactivity at 1 and 4 h after treatment, respectively (Fig. 5), and ~45% of the plasma radioactivity from 0–24 h (Table 1). The remaining radioactivity is presumed to be accounted for by radioactivity associated with MTIC, AIC, the further breakdown of these to unidentified degradation products as a result of AIC being an intermediate in purine biosynthesis and uric acid synthesis (22–25), and a negligible amount to the formation of TMA. No radioactivity at 1 and 4 h was associated with MTIC or TMA. This indicates that MTIC is rapidly converted to AIC in plasma, and metabolism of TMZ to TMA plays a negligible role in TMZ elimination, which has been described elsewhere (5, 13, 15, 16).

After administration of 200 mg of 14C-TMZ, drug-derived radioactivity was excreted principally in the urine. Metabolite profiling of the total radioactivity excreted in the urine revealed that TMZ was eliminated renally as unchanged drug (5.5%), AIC (12%), TMA (2.3%), and unidentified polar compounds (17%; Table 3). These results are in good agreement with those of Dhodapkar et al. (5), who reported that minimal amounts of the administered TMZ dose were excreted as parent compound (5.6%) and TMA (0.8%). However, an assessment of the combined cumulative excretion of radioactivity in the urine (38%) and feces (<1%) out to 14 days after treatment indicates that recovery of radioactivity was incomplete. Because AIC is an intermediate in purine biosynthesis (22, 24, 25), the incomplete recovery of radioactivity is likely due to the incorporation of AIC into the urine pool. Incorporation of AIC into the purine biosynthetic pathway has been demonstrated in a female volunteer administered 14C-AIC (23). Analysis of urinary metabolites confirmed the presence of radiolabeled AIC, uric acid, xanthine, hypoxanthine, guanine, adenine, and 7-methylguanine. The unidentified polar metabolites measured in urine after administration of 14C-TMZ could represent products formed by incorporation and metabolism of AIC in the purine and uric acid biosynthesis pathways. In addition, a small portion of the radioactivity may also have been eliminated as 14C02.

The metabolic pathway for TMZ is a pH-dependent reaction, which begins with the conversion of TMZ to MTIC, and then subsequent breakdown of MTIC to AIC. In the present study, TMZ was rapidly eliminated with a mean half-life value of 1.9 h (Table 1). Systemic exposure to MTIC was lower than that for TMZ (~2.2%), and the elimination of MTIC paralleled TMZ, which resulted in a similar apparent half-life value of 2.1 h (Table 1). These observations suggested that the appearance of MTIC concentrations in plasma were formation rate-limited (21). That is, the formation of MTIC from TMZ, and not the degradation of MTIC to AIC, is the rate-determining step characterizing MTIC disposition. Formation-rate limited kinetics apply when: (a) the elimination rate constant of the parent compound is smaller than that of the metabolite; (b), metabolite concentrations decline in parallel with levels of the parent drug; and (c) the metabolite is cleared so rapidly that levels of metabolite remain lower than parent compound (if they both have the same volume of distribution). It has been demonstrated that MTIC concentrations decline in parallel with TMZ and that MTIC represents a small (2.2%) percentage of systemic exposure to parent compound in this study and elsewhere (13, 16). However, the in vivo rate constants for conversion of TMZ to MTIC and MTIC to AIC have not been characterized in human plasma.

In this study, a pharmacokinetic model that describes the kinetics of conversion of TMZ to MTIC and the subsequent degradation of MTIC to AIC was developed (Fig. 2). On the basis of data from mechanistic and preclinical studies (7–9), the minimal renal excretion of parent compound (5–6%), and the negligible metabolism of TMZ to TMA (1 to 2%) observed in this and other phase I studies (5, 15), the model described TMZ clearance principally via the pH-dependent chemical conversion to MTIC, TMZ, MTIC, and AIC concentrations were well described by the pharmacokinetic model, and TMZ pharmacokinetic values were in excellent agreement with those obtained using model-independent methods (Table 1). The rate constant of TMZ elimination (0.39 h−1) was smaller than that for MTIC (17 h−1), which supports that a formation-rate limited kinetic process describes MTIC disposition. The half-life for MTIC, calculated from the rate constant of MTIC elimination, averaged 2.5 min, which is similar to that observed when TMZ is placed in physiological solutions (9). Interpatient variability in TMZ
clearance, via the formation of MTIC, was low (~20%) and comparable with values reported previously (5). The potential clinical importance of this observation is that the generation of MTIC, the active moiety, should be relatively consistent between patients. Indeed, the pharmacokinetic model presented here, provides evidence that pH-dependent chemical conversion to MTIC is the principal elimination pathway for TMZ.

In conclusion, oral TMZ was rapidly absorbed and eliminated, and the principal route of TMZ clearance was via the pH-dependent formation of MTIC, with renal excretion playing a minor role in TMZ elimination. The observed low interpatient variability in TMZ clearance suggests that TMZ administration will result in efficient generation of the active metabolite MTIC in all tissues that TMZ is present, which supports a therapeutic advantage for TMZ. It is anticipated that interpatient variation in drug effect (toxicity and/or efficacy) may not reflect differences in TMZ exposure, or generation of MTIC, but in differences at the tissue or cellular level, such as varying levels of the O\textsuperscript{6}-alkylguanine-DNA alkyltransferase protein, which repairs the methylguanine lesions. Future studies will define relevant cellular-specific parameters that are associated with drug effect.

ACKNOWLEDGMENTS

We thank Mary Duerr for assistance with study coordination and data collection, Yelena Zabelina for assistance with sample processing, and the referring medical staff and medical and nursing staffs of The Johns Hopkins Oncology Center for the care of the patients in this study.

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Absorption, Metabolism, and Excretion of $^{14}$C-Temozolomide following Oral Administration to Patients with Advanced Cancer

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