Schedule-dependent Activity of Temozolomide plus CPT-11 against a Human Central Nervous System Tumor-derived Xenograft


 Departments of Surgery [V. J. P., S. K., S. P. J., H. S. F.], Pathology [D. D. B., H. S. F.], and Pharmacology [G. B. E.], Duke University Medical Center, Durham, North Carolina 27710; Department of Molecular Pharmacology, St. Jude Children’s Research Hospital, Memphis, Tennessee 38105 [P. J. H.]; Department of Medicine, University of Chicago, Chicago, Illinois 60637 [M. E. D.]; and Department of Cellular and Molecular Physiology, The Milton S. Hershey Medical Center, Hershey, Pennsylvania 17033 [A. E. P.]

ABSTRACT

Temozolomide, an imidazole tetrazinone, and CPT-11, a camptothecin derivative, have previously been shown to have anti-central nervous system tumor activity in laboratory and clinical studies. The current experiments were designed to evaluate the activity of temozolomide plus CPT-11 against a malignant glioma-derived xenograft, D-54 MG, growing s.c. in athymic nude mice. The initial schedule of i.p. drug administration was temozolomide at 0.1 LD10 on day 1 and CPT-11 at 0.1 LD10 on days 1–5 and 8–14. The combination of these two agents produced greater than additive activity against D-54 MG. This enhanced activity was maintained when the initial administration of CPT-11 was delayed to day 3 or day 5. However, when CPT-11 was administered first on day 1 using 0.5 LD10 (for the single dose schedule) followed by temozolomide (0.1 LD10) 5 h, 3 days, or 5 days later, the enhancement of activity was substantially reduced. These results demonstrate that the combination of temozolomide plus CPT-11 displays a schedule-dependent enhancement of antitumor activity, suggest a mechanistic explanation for the enhanced activity, and provide the rationale for a Phase I trial of this regimen.

INTRODUCTION

The outcome for patients with malignant glioma remains dismal, with the vast majority of patients dying within a short time after diagnosis (1). Treatment of the primary tumor site with surgery and radiotherapy fails to control local disease and fails to address tumor cells that invariably migrate to distant locations within the brain. Despite the excitement of newer strategies such as cancer vaccines or gene therapy, few, if any, patients have actually benefited from these interventions (2). Accordingly, progress, at least in the next several years, will require improvements in chemotherapy.

Recent laboratory and clinical studies have confirmed the activity of temozolomide and CPT-11, respectively, in the treatment of malignant glioma (3–6). Temozolomide is an imidazole tetrazinone that transfers a methyl group to three sites, N2-guanine, N3-adenine, and O6-guanine (7). The toxic lesion is felt to be the O6-guanine adduct, which leads to a lethal cycle of DNA mismatch repair if the adduct is not removed by AGT.4 CPT-11 is a camptothecin derivative that produces antitumor activity by inhibition of topoisomerase I (8).

Previous studies have shown enhanced and schedule-dependent antiglioma activity when CPT-11 was combined with BCNU (9, 10). The mechanism for this enhanced activity remains unclear but may reflect the presence of the BCNU-induced chloroethyl adduct on the O6-position of guanine. Accordingly, we evaluated the interaction between CPT-11 and temozolomide to define the activity and schedule dependency of this combination.

MATERIALS AND METHODS

Animals. Male and female athymic BALB/c mice (nu nu genotype; 6 weeks of age or older) were used for all studies and were maintained as described previously (11).

Xenografts. A human malignant glioma-derived xenograft, D-54 MG, was used for in vivo studies. The xenograft was maintained as described previously (12).

Drugs. Temozolomide was provided by Schering-Plow Research Institute (Kenilworth, NJ). CPT-11 was provided by Pharmacia & Upjohn Global Distribution Center (Kalamazoo, MI). Procarbazine was provided by the Pharmaceutical Research Branch of the National Cancer Institute (Bethesda, MD).

s.c. Xenograft Transplantation. s.c. tumor transplantation was performed in the right flank of the animals with an inoculation volume of 50 μl using a brei prepared from xenografts (13).

Tumor Measurements. Tumors were measured twice weekly with hand-held Vernier calipers (Scientific Products, Chicago, Illinois). Tumors were measured twice weekly with hand-held Vernier calipers (Scientific Products, Chicago, Illinois).

4The abbreviations used are: AGT, O6-alkylguanine-DNA alkyltransferase; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea.

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2Dr. Gertrude B. Elion died on February 21, 1999 during the completion of this work.

3To whom requests for reprints should be addressed, at Department of Surgery, Box 3624, Duke University Medical Center, Durham, NC 27710. Phone: (919) 684-5301; Fax: (919) 681-1697.

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McGraw, IL). Tumor volume was calculated according to the following formula: \( \frac{\text{width}^2 \times \text{length}}{2} \).

**Xenograft Therapy.** Temozolomide was given via i.p. injection at a dose of 52.5 or 105 mg/m² (17.5 or 35 mg/kg) in 10% DMSO in saline on days 1, 3, or 5, which represents one-twentieth or one-tenth of the dosage lethal to 10% of treated animals. CPT-11 was given via i.p. injection at a dose of 12 mg/m² (4 mg/kg) in 10% DMSO in saline on days 1–5 and 8–12, days 3–7 and 10–14, or days 5–8 and 12–16, which represents one-tenth the dosage lethal to 10% of treated animals. In addition, a single treatment of CPT-11 at a dose of 1101 mg/m² (367 mg/kg) in 10% DMSO in saline on day 1 was administered, followed immediately by temozolomide. Procarbazine was given at a dose of 600 mg/m² (200 mg/kg) in saline on day 1. Groups of 10 randomly selected mice began receiving treatment when the tumor volume was in the range of 100–500 mm³ (10–15 days after tumor implantation) and were compared with control animals receiving drug vehicle.

**Assessment.** The response of the s.c. xenografts was assessed by delay in tumor growth and by tumor regression. Growth delay, expressed as \( T - C \), is defined as the difference in days between the median time required for tumors in treated \((T)\) and control \((C)\) animals to reach a volume five times greater than that measured at the start of treatment. Tumor regression is defined as a decrease in tumor volume over two successive measurements. Statistical analyses was performed using a personalized SAS statistical analysis program, the Wilcoxon rank order test for growth delay, and Fisher’s exact test for tumor regression as described previously (14).

**RESULTS**

**Toxicity.** The toxic deaths and mean nadir weight loss secondary to drug treatment are indicated in Table 1.

**Activity.** The initial set of studies was conducted with CPT-11 administered on days 1–5 and 8–12 and temozolomide administered on day 1. The combination of CPT-11 and temozolomide on this schedule produced a substantial increase in antitumor activity compared with the agents used alone. Delay of the start of CPT-11 to day 3 or day 5 did not alter this enhanced activity (Table 1). The second set of studies was conducted with CPT-11 administered as a single dose on day 1, followed immediately by temozolomide on day 1, 3, or 5. The activity of CPT-11 plus temozolomide in combination was dramatically decreased when CPT-11 preceded temozolomide. The final set of studies, which replaced temozolomide with procarbazine administered on day 1 followed by CPT-11 administered on days 1–5 and 8–12, did not result in an increase in activity.

The enhancement index, defined as the growth delay produced by combination therapy of two agents divided by the sum of the growth delays of the two agents, was highest for temozolomide administered on day 1 followed by CPT-11 administered on days 1–5 and 8–12, days 3–7 and 10–14, or days 5–9 and 12–16, with values from 1.3–1.5 (Table 1). Treatment with CPT-11 followed by temozolomide produced an enhancement index of 0.8–1.1. Treatment with procarbazine plus CPT-11 only produced an enhancement index of 1.0–1.1.

**DISCUSSION**

Cancer remains a scourge for which few effective therapeutic options are available. Treatment with surgery and radiotherapy, although effective for a few cancers such as early-stage Hodgkin’s disease and neuroblastoma, is inadequate for most malignancies, including almost all brain tumors. Chemotherapy is an important modality, but its effectiveness is limited by de novo or acquired resistance in the majority of cancers (15). The promise of newer therapies such as gene therapy or vaccines, despite almost continuous celebration in the lay press, remains unfulfilled (2). Accordingly, new strategies for treatment of advanced or refractory malignancies, at least for the next 3–5 years, will require advances in the use of chemotherapeutic agents, including rational combinations reflecting promising preclinical interactions.

Temozolomide is a methylating agent that has been shown to be active in the treatment of a spectrum of brain tumors in preclinical and clinical studies (2, 6, 16, 17). The mechanism of action of this agent is methylation at a number of sites including the critical \( O^6 \) position of guanine, which is felt to be the cytotoxic lesion. This leads to incorrect incorporation during replication of bases during replication in which an \( O^6 \)-methylguanine is recognized as an adenine and paired with thymine. This initiates a cellular response to repair this mismatch by removal of the thymine. However, methylguanine is again paired with thymine, and an ultimately lethal cycle of mismatch repair continues. CPT-11 is a camptothecin derivative, which has been shown to be similarly active against central nervous system tumor xenografts and patients with recurrent malignant glioma (4, 5). CPT-11 produces antitumor activity by inhibition of topoisomerase I (8), in effect stabilizing the topoisomerase I-initiated cleavage complexes. The rationale for the combination of these two agents was based on several foundations, particularly our prior work combining CPT-11 and BCNU (9, 10).

CPT-11 and BCNU were shown to produce enhanced activity when given in combination in the treatment of a malignant glioma-derived xenograft in athymic nude mice (9, 10). The combination of BCNU and CPT-11 produced a marked increase in antitumor activity compared with the two agents used alone that was clearly greater than additive. Moreover, the increase in activity was absolutely schedule dependent, with the highest enhancement of activity seen when BCNU was given on day 1 and CPT-11 was given on days 1–5 and 8–12. Delay of CPT-11 to day 3 or 5 or delay of BCNU to day 8 substantially reduced the enhanced activity. These results suggested to us that the presence of a BCNU-induced adduct or a cross-link before administration of CPT-11 was critical for enhanced activity (and led to a current trial of BCNU plus CPT-11 in patients with recurrent malignant glioma). The BCNU monoadduct forms initially at the \( O^6 \) position of guanine, which suggested to us that another agent that placed an adduct at the \( O^6 \) position of guanine but did not result in the formation of a cross-link might resolve the question of whether a monoadduct or a cross-link was the critical lesion responsible for the enhanced antitumor activity. Temozolomide produces the monoadduct \( O^6 \)-methylguanine and was chosen for these current studies (7). Temozolomide given in combination with CPT-11 produced a marked increase in activity compared with the two agents used alone, which strongly suggests that the critical lesion is an adduct at the \( O^6 \).
position of guanine and not a cross-link. The lack of increased activity when procarbazine was used with CPT-11 may reflect differences in the time course of adduct formation or a mutual interference of procarbazine/CPT-11 metabolism.

Our current studies also demonstrate the schedule dependency of the interaction between temozolomide and CPT-11. Unlike prior work with BCNU and CPT-11 in which delay of CPT-11 decreased the enhanced activity, presumably due to conversion of the monoadduct to a DNA interstrand cross-link, delay of CPT-11 following temozolomide does not decrease

### Table 1: Treatment of nude mice bearing D-54 MG xenografts growing s.c. with temozolomide, procarbazine, CPT-11, temozolomide plus CPT-11, or procarbazine plus CPT-11

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<th>Day of procarbazine</th>
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<th>Enhancement index</th>
<th>Regressions</th>
<th>Toxic deaths</th>
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a \( T - C \), the difference in days between the median time for treated \( T \) and control \( C \) animals to reach a volume five times greater than that measured at the start of treatment.

b Enhancement index is defined as the growth delay \( (T - C) \) produced by combination therapy with temozolomide or procarbazine plus CPT-11 divided by the sum of the growth delays of temozolomide or procarbazine plus CPT-11 \( (T - C \) of temozolomide or procarbazine plus \( T - C \) of CPT-11).

c Tumor regression is defined as a decrease in tumor volume over two successive measurements (number of complete regressions is in parentheses).

d Temozolomide was administered i.p. on the day indicated at a dose of 35 mg/kg in 10% DMSO.

e CPT-11 was administered i.p. on the day indicated at a dose of 4 mg/kg in 10% DMSO.

f Procarbazine was administered i.p. on the day indicated at a dose of 200 mg/kg in saline.

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activity. It is unlikely that the O6-methylguanine would be removed during the delay before administration of CPT-11 because the xenograft studied, D-54 MG, does not demonstrate measurable AGT. Conversely, administration of CPT-11 followed by temozolomide produced no enhancement of activity. This strongly implies that the mechanism responsible for enhanced activity of these two agents is placement of an adduct at the O6 position of guanine before administration of CPT-11. Whereas temozolomide-induced adducts would be expected to persist over 5 days in the absence of AGT, BCNU-induced monoadducts are converted to cross-links, with peak formation at 12 h (18). These cross-links may not be capable of enhancing the activity of subsequently administered CPT-11.

Recent work suggests a mechanism for this enhanced activity of CPT-11 when administered after temozolomide or BCNU. Pourquier et al. (19) demonstrated that O6 alkylation of guanine induces topoisomerase I-DNA covalent complexes in vitro and in N-methyl-N'-nitro-N-nitrosoguanidine-treated Chinese hamster ovary cells. This increase in topoisomerase I cleavage complexes would be expected to increase cellular sensitivity to topoisomerase I inhibitors, including CPT-11. Sekikawa et al. (20) demonstrated that AGT is a critical determinant of cytotoxicity for topoisomerase I inhibitors. CPT-11 cytotoxicity correlated with AGT gene expression and was increased by O6-alkylguanine-mediated depletion of AGT. Together, this work suggests that O6 alkylation with temozolomide or BCNU is the mechanism responsible for enhanced antitumor activity when these agents are administered before CPT-11.

Studies combining CPT-11 plus temozolomide and CPT-11 plus BCNU warrant further exploration in other malignancies because this interaction is likely to be histology independent. Recent work by Houghton et al. has demonstrated the enhanced activity of temozolomide plus CPT-11 in neuroblastoma, rhabdomyosarcoma, and glioblastoma xenografts independent of AGT or mismatch repair activity. Furthermore, clinical trials building on this interaction may be facilitated by the nonoverlapping toxicities of these agents. We have opened a Phase I trial of CPT-11 plus temozolomide for patients with recurrent malignant glioma that may allow a precise clinical translation of these preclinical studies.

REFERENCES

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