The Emerging Role of Irinotecan (CPT-11) in the Treatment of Malignant Glioma in Brain Tumors

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Irinotecan in Brain Tumors

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Irinotecan is a water-soluble derivative of camptothecin, an alkylator originally extracted from the Chinese tree *Camptotheca acuminata*. Laboratory studies have demonstrated the activity of irinotecan in a broad panel of pediatric and adult central nervous system tumor xenografts in athymic nude mice. These studies led to a Phase II trial that confirmed the activity of this agent in the treatment of recurrent malignant glioma. Subsequent laboratory studies have demonstrated that a combination of irinotecan (CPT-11) and alkylating agents, particularly 1, 3-bis(2-chloroethyl)-1-nitrosourea (BCNU), increases antitumor effects to a level well above the additive effects of the individual agents. These laboratory studies generated a recently completed Phase I trial of CPT-11 + BCNU, which now is being evaluated in a formal Phase II trial for adults with newly diagnosed or recurrent malignant glioma. More recent studies have demonstrated similar interaction between CPT-11 and temozolomide and have led to a Phase I trial of these agents in the treatment of adults with malignant glioma. Studies currently are addressing the role of O6-alkylguanine-DNA alkyltransferase (AGT) in reducing the benefits of combining CPT-11 with temozolomide and the potential therapeutic gain from utilizing an inhibitor of AGT.

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Malignant glioma has remained a major cause of death in adults and children despite therapeutic strategies that include maximal surgical resection followed by radiotherapy. Adjuvant chemotherapy is employed routinely for children and frequently for adults, but tumors almost invariably recur at the primary site and also display distant spread. These findings provide a strong rationale for improvements in local and disseminated disease. Accordingly, efforts to define chemotherapeutic agents that are active in the treatment of malignant glioma are critically needed. Although there is great promise for future strategies that involve anti-angiogenic agents, chemotherapy, or vaccines, it likely will be years before we see true therapeutic benefits from these newer modalities. There is little doubt that chemotherapy will be needed for the foreseeable future, and it is clear that better agents are required if we are to have an impact on the treatment of malignant glioma.

Preclinical Evaluation of Irinotecan (CPT-11) in Central Nervous System Tumor Xenografts

Irinotecan (Camptosar; Pharmacia Corporation, Peapack, NJ) is a water-soluble chemical derivative of camptothecin, an alkylator originally extracted from the Chinese tree *Camptotheca acuminata*. Camptothecin and its derivatives inhibit topoisomerase I, an enzyme essential for DNA transcription, replication, and repair. The original
observation of the activity of CPT-11 in the treatment of pediatric and adult central nervous system (CNS) tumor xenografts was published in 1997. CPT-11 was evaluated against a human tumor xenograft model that was derived from adult and pediatric central nervous malignancies. The panel of tumors included childhood high-grade gliomas (D-212 MG and D-456 MG), adult high-grade gliomas (D-54 MG and D-245 MG), medulloblastomas (D341 Med and D487 Med), ependymomas (D528 EP and D612 EP), and a rhabdomyosarcoma (TE-671), as well as sublines that demonstrated resistance to busulfan (D-456 MG [BR]), cyclophosphamide (TE-671 CR), procarbazine (D-245 MG [PR]), or melphalan (TE-671 MR). Tumors were implanted and grown subcutaneously and intracranially in athymic nude mice. In replicate experiments, CPT-11 was administered at 40 mg/kg per dose (the dosage lethal to 10% of treated animals [LD10]) via intraperitoneal injection in 10% dimethylsulfoxide on Days 1–5 and 8–12. Animals that had received CPT-11 demonstrated statistically significant (P < 0.001) growth delays in all subcutaneous xenografts that were tested, including those that were resistant to busulfan, cyclophosphamide, procarbazine, and melphalan; growth delay ranged from 21.3 days in D487 Med to > 90 days in several other tumor lines. Tumor regression also was evident in all treated animals that bore subcutaneous tumors, with some xenografts exhibiting complete tumor regression. Animals treated with CPT-11 demonstrated statistically significant (P < 0.001) increases in survival for the 2 intracranial xenografts—D341 EP (73.0% increase) and D-456 MG (114.2% increase). In this study of over 40 drugs that were evaluated, CPT-11 was the most active agent against central nervous system xenografts; we believe that it should be advanced to clinical trial as quickly as possible.

CPT-11 Therapy for Adults with Recurrent or Progressive Malignant Glioma

The treatment results (detailed above) in CNS tumor xenografts led to the design and initiation of a Phase II trial in adults with progressive or recurrent malignant gliomas. Patients with progressive or recurrent malignant gliomas were enrolled on study from October 1996 to August 1997. CPT-11 was administered at a dose of 125 mg/m² via a 90-minute intravenous (IV) infusion once weekly for 4 weeks followed by a 2-week rest, which constituted 1 course of treatment. Plasma concentrations of CPT-11 and its active metabolites, SN-38 and SN-38 glucuronide (SN-38G), were measured in a subset of patients. All 60 accrued patients (36 males and 24 females) were treated with CPT-11 and were assessable for toxicity, response, and survival; pharmacokinetic data were available in 32 of the patients. Nine patients (15%; 95% confidence interval, 6–24%) exhibited a partial response, and 33 (55%) achieved stable disease that exceeded 2 courses (12 wks). Observed toxicity was limited to infrequent neutropenia, nausea, vomiting, and diarrhea. In terms of pharmacokinetics, respective CPT-11, SN-38, and SN-38G areas under the plasma concentration vs. time curves through infinite time values for the observed patients were approximately 40%, 25%, and 25% of those previously observed in metastatic colorectal cancer patients who did not receive antiepileptics or chronic dexamethasone treatment. Thus, CPT-11, when administered at a standard starting dose and on a standard treatment schedule, exhibits significant activity in patients with recurrent malignant glioma; however, the observed low incidence of both severe toxicity and plasma concentrations of CPT-11 and SN-38 suggested that concurrent treatment with anticonvulsants and dexamethasone enhances drug clearance.

These results indicated that it was critical that a Phase I trial be defined for patients who were receiving cytochrome p450-inducing anticonvulsants, specifically phenobarbital, carbamazepine, and hydantoin. The study was conducted under the auspices of the National Cancer Institute and revealed that, in patients who concomitantly were treated with anticonvulsants, the maximal tolerated dose was 410 mg/m² administered weekly for 4 weeks followed by a 2-week break. Current studies are aimed at evaluating the activity of CPT-11 at this dose in patients with brain tumors who are receiving anticonvulsants.

Preclinical Evaluation of Interactions between CPT-11 and Alkylating Agents

We subsequently examined the antitumor effects of the combination of CPT-11 with each of 3 alkylating agents: 1, 3-bis(2-chloroethyl)-1-nitrosourea (BCNU), busulfan, and cyclophosphamide. In replicate experiments, each combination was assayed against a panel of human tumor xenografts that were derived from central nervous system malignancies, including adult high-grade gliomas (D-54 MG and D-245 MG) and a childhood ependymoma (D-612 EP). The alkylating agents were administered on Day 1 at doses varying from 10–75% of LD10 and CPT-11 was administered on Days 1–5 and 8–12 at doses ranging from 10–100% of LD10. Antitumor effects ranged from those that were less than additive (7.61 days less than additive with 0.5 LD10 CPT-11 + 0.75 LD10 cyclophosphamide in D-54 MG) to statistically significant (P < 0.001) supra-additive effects (18.80 days greater than additive with 0.5 LD10 CPT-11 + 0.5 LD10 BCNU in D-54 MG). The
results of this study demonstrate that in some cases, combined use of the topoisomerase 1 inhibitor CPT-11 and an alkylating agent, particularly BCNU, may increase the antitumor effect of the treatment to a level well above additive with no increase in host toxicity (0/10 deaths in both experiments cited above). The data also reinforce the idea that this combination should be considered for use in combination chemotherapy of central nervous system malignancies.

Phase I Trial of CPT-11 + BCNU
The interaction between CPT-11 and BCNU in the mouse experiments led us to design and initiate a Phase I trial of CPT-11 + BCNU in patients with recurrent malignant glioma. CPT-11 is a topoisomerase I inhibitor previously shown to be active in the treatment of malignant glioma (MG). CPT-11 has attracted much attention as a new type of cancer chemotherapeutic agent due to its unique mechanism of action, the inhibition of DNA topoisomerase I (an enzyme thought to be necessary for DNA replication). Furthermore, treatment of human MG xenografts in athymic nude mice with CPT-11 and BCNU revealed enhanced activity. This finding suggested that a combination of these two agents may produce enhanced antineoplastic activity against human glioma and provided the rationale for the Phase I trial that we have conducted. Patients were stratified into anticonvulsant (hydantoin, carbamazepine, or phenobarbital) or no anticonvulsant use groups. BCNU was administered at a starting dose of 100 mg/m² over a 1-hour period every 6 weeks on the same day as the first irinotecan dose (i.e., Wks 1, 7, etc.). CPT-11 was administered intravenously over the course of 90 minutes once weekly. A treatment cycle consisted of 4 weekly administrations of CPT-11 followed by a 2-week rest. Every effort was made to eliminate delays between treatments. Starting at 25 mg/m², the CPT-11 dosage escalated in cohorts of 3–6 patients. Sixty-one patients have been treated (44 with anticonvulsant use and 17 with no anticonvulsant use). The maximal tolerated dose (MTD) for patients who were not receiving anticonvulsants was 125 mg/m². The MTD for patients who were receiving anticonvulsants was 225 mg/m². Myelosuppression was the predominant form of toxicity observed.

Phase II Trial of BCNU + CPT-11 in Adults With Newly Diagnosed or Recurrent Malignant Glioma
We currently are conducting a Phase II trial of CPT-11 + BCNU utilizing the CPT-11 dose as defined in the Phase I trial for adults with newly diagnosed or recurrent malignant glioma. Mild myelosuppression and mild to moderate diarrhea have been the only observed toxicities in the trial. Results are preliminary, and responses have been observed in adults with both newly diagnosed and recurrent malignant glioma.

Preclinical Evaluation of the Mechanism of Interaction Between CPT-11 and BCNU
We subsequently evaluated the schedule dependence of the CPT-11 + BCNU regimen in the treatment of the malignant glioma xenograft D-54 MG. The combination of CPT-11 and BCNU resulted in the highest enhancement index (2.0–3.3) when BCNU was administered on Day 1 and CPT-11 was given on Days 1–5 and 8–12. When CPT-11 administration was delayed until Day 3 or 5, substantially decreased activity with enhancement indices of 1.6–1.8 and 0.6–1.0, respectively, was observed; delaying BCNU administration until Day 8 similarly reduced CPT-11 activity (enhancement indices, 1.2–1.4). These data strongly suggested that CPT-11 was inhibiting topoisomerase I, which might mediate repair of BCNU-induced crosslinks. However, we recognized that inhibition of topoisomerase I might not be affecting cross-link repair and that the placement of an adduct at the O⁶ position of guanine, which is a prelude to the formation of BCNU-induced interstrand crosslinks, might in some way be responsible. Accordingly, studies were conducted using a methylation agent that would place an adduct at the O⁶ position of guanine but not lead to formation of an interstrand crosslink.

Preclinical Evaluation and the Interaction Between CPT-11 and Temozolomide
Experiments were designed to evaluate the activity of CPT-11 + temozolomide against a xenograft that was derived from a malignant glioma, D-54 MG, and grown subcutaneously in athymic nude mice. The initial schedule of intraperitoneal 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. This combination produced supra-additive activity against the xenograft, and the enhanced activity was maintained even when the initial CPT-11 administration was delayed until Day 3 or 5. However, when CPT-11 was given first on Day 1 at 0.5 LD₁₀ (for a single-dose schedule) followed by temozolomide (0.1 LD₁₀) 5 hours, 3 days, or 5 days later, a substantial reduction in the enhancement of activity was observed. These results demonstrate that a combination of CPT-11 + temozolomide leads to schedule-dependent enhancement of antitumor activity and suggest an explanation for the enhanced activity; the results also provide the rationale for a Phase I trial of this combination.

Sekikawa et al. demonstrated that O⁶-alkylguanine-DNA alkyltransferase (AGT) is a critical determi-
nant of cytotoxicity for topoisomerase I inhibitors. CPT-11 cytotoxicity against Chinese hamster ovary cells was found to be inversely correlated with AGT gene expression and was enhanced by O6-alkylguanine (BG)-mediated depletion of AGT. Recent work suggests a mechanism for this enhanced activity of CPT-11 when it is administered after temozolomide or BCNU. Pourquier and colleagues demonstrated that O6-alkylation of guanine induces topoisomerase I-DNA covalent complexes in vitro and in N-methyl-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. Together these studies suggest that O6-alkylation by temozolomide or BCNU is the mechanism responsible for enhanced antitumor activity when these agents are administered before CPT-11.

Phase I Trial of CPT-11 + Temozolomide

The results detailed above provided the rationale for a Phase I trial of CPT-11 + temozolomide. Temozolomide is administered as a single dose at 200 mg/m2 on Day 1. CPT-11 subsequently is administered weekly for 4 weeks followed by a 2-week rest. CPT-11 is dose-escalated until a maximal tolerated dose has been achieved. This trial is designed to bring about methylation of tumor DNA prior to treatment with the topoisomerase I inhibitor. Studies are in progress, and the maximal tolerated dose of CPT-11 has not yet been achieved.

Preclinical Evaluation of the Interaction Between CPT-11, Temozolomide, and O6-BG

The interaction between CPT-11 and temozolomide strongly suggests that temozolomide-induced methyl groups must remain at the O6 position of guanine tumor DNA in order for the activity of CPT-11 to be enhanced. However, the majority of tumors exhibit high expression of O6-alkylguanine-DNA alkyltransferase and may remove the adduct prior to CPT-11-mediated cytotoxicity. Accordingly, we are currently evaluating the interaction between CPT-11 and temozolomide when O6-benzylguanine is also administered. O6-BG is a substrate for AGT and leads to an irreversible inactivation of this protein. Temozolomide brings about the methylation of the O6 position of guanine, and O6-BG prevents removal of the methyl adduct by AGT. As a result, CPT-11 is given additional time to produce cytotoxic effects in tumor cells whose DNA has been methylated. Clinical studies were conducted on the AGT-positive malignant glioma D-456 MG grown in athymic nude mice. The use of temozolomide results in a growth delay of 2.9 days, whereas the use of temozolomide + O6-BG results in a growth delay of 7.3 days. Treatment with CPT-11 produces a growth delay of 23.7 days, with the addition of O6-BG only increasing the growth delay to 26.1 days. The combination of CPT-11 + temozolomide produced the expected marked increase in antitumor activity, with an observed growth delay of 43.2 days. However, the combination of CPT-11, temozolomide, and O6-BG produced an apparent cure with growth delays of greater than 150 days.

These results suggest that Phase II trials of CPT-11 + temozolomide are clearly warranted. Furthermore, a Phase I trial of CPT-11 + temozolomide + O6-BG will be important if AGT-mediated removal of methyl adduct groups diminishes the benefit of the interaction between CPT-11 and temozolomide.

REFERENCES


