Synergistic Antitumor Activity of Irinotecan in Combination with 5-Fluorouracil in Rats Bearing Advanced Colorectal Cancer: Role of Drug Sequence and Dose

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Abstract

The basis for current clinical trials in the treatment of colorectal cancer with the combination of irinotecan (CPT-11) and 5-fluorouracil (FUra) with or without leucovorin (LV) is their proven activity as single agents, their different mechanisms of action, and lack of CPT-11 cross-resistance to previous FUra/LV treatment. The role of drug dose and administration sequence in this combination was studied in vivo using a rat colon tumor model (Ward colon carcinoma); we administered CPT-11 and FUra by i.v. push once a week for four consecutive weeks (weekly × 4), a clinically relevant schedule. The maximum tolerated doses (MTDs) of CPT-11 and FUra administered as single agents were 100 mg/kg/week for both agents. Three different combination administration sequences were evaluated: (a) CPT-11 administered simultaneously with FUra (sequence I); (b) FUra administered 24 h before CPT-11 (sequence II); and (c) CPT-11 administered 24 h before FUra (sequence III). When combining the two drugs at 50% of their respective MTD, the antitumor efficacy was sequence dependent with 62, 38, and 95% complete tumor regression rate for sequences I, II, and III, respectively. For sequences I and II, dose escalation to 75% of the MTD for each drug was paralleled by reversible host toxicity with no significant increase in the antitumor activity of the combination. With sequence III, however, the combination was lethal in 100% of treated animals when the doses of both drugs were at 75% of the MTD or higher. With the sequential combination of CPT-11 followed 24 h later by FUra (sequence III), the high complete tumor regression rate (cure) could be maintained, even when the dose of CPT-11 was reduced to 12.5% of the MTD as long as the doses of FUra was kept at 50–75% of the MTD. The data demonstrate that the antitumor activity and toxicity of combining CPT-11 with FUra is highly sequence dependent and that a sequence of CPT-11 preceding FUra is superior with a significant increase in the therapeutic index over the other sequences tested. In addition, the data also demonstrate that toxicity associated with high dose of CPT-11 can be eliminated without loss of the antitumor efficacy by reducing the dose of CPT-11 to at least 50% of its MTD, whereas the dose of FUra is kept at 50–75% of its MTD.

Introduction

Although surgical resection is the primary treatment modality for colorectal cancer, FUra-based chemotherapy plays an important role with proven benefits with regard to relapse-free survival in adjacent colorectal cancer (1). FUra exhibits only limited efficacy when used as a single agent with objective tumor responses from 7 to 17% and a median survival time <1 year (2). Although biomodulation of FUra by LV increased tumor response rate in patients with advanced colorectal cancer, no significant survival benefit has been observed (2–4). Thus, there is still a need for improvement of the chemotherapeutic treatment of colorectal cancer.

Irinotecan (CPT-11) is a water-soluble, semisynthetic derivative of CPT, which is converted in vivo to its active metabolite, SN-38. CPT-11 is active clinically in treatment of colorectal cancer with no cross-resistance to prior therapy of FUra with or without LV modulation, with reported response rates of 15–32% in chemo-naive and 18–27% in FUra-pretreated patients (5–8). The primary mechanism of action of CPT-11 is stabilizing the complex between DNA and topoisomerase I, a nuclear enzyme that facilitates DNA replication and transcription (9, 10). Collisions of advancing DNA replication forks with these stabilized complexes, through a cascade of events, lead to formation of single- and double-strand DNA breaks and ultimately to cell death (9, 10).

The combination of CPT-11 with FUra-based regimens (with or without LV) with different schedules has been evaluated clinically in patients with colorectal cancer (11, 12). Although antitumor response was not a primary objective in these dose-finding studies, objective responses were noted with manageable toxicity (11, 12). Recently, Vanhoef et al. (12) reported 64% objective responses in 25 patients with metastatic colorectal cancer using a weekly schedule of CPT-11 together with FUra plus LV as first-line chemotherapy. Preclinical studies to define the optimal sequence of drug administration were inconclusive, with most reports favoring a sequence in which CPT-11 was given before FUra (13, 14) as well as reports that showed that the reverse sequence was equally as effective (15).

In this study, we evaluated the role of administration sequence and drug dose in the combination of CPT-11 with FUra on antitumor activity and therapeutic selectivity using an i.v. push weekly × 4 schedule in rats bearing advanced colorectal carcinoma.

Materials and Methods

Animals and Tumors. Female Fisher 344/N rats (body weight, 150–180 g), 8–12 weeks of age, were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN) and kept four rats/cage with water and food ad libitum according to an institutional approved animal protocol. The chemically induced Ward colorectal carcinoma was used in this study (16). Nonnecrotic tumor pieces (0.1 g) were transplanted s.c. via trocar under slight ether anesthesia.

Drugs. CPT-11 was supplied by Pharmacia & Upjohn Company (Kalama-zoo, MI) as a ready-to-use clinical formulation solution in 5-ml vials that contained 100 mg of drug (20 mg/ml). FUra was purchased from Hoffmann-La Roche, Inc. (Nutley, NJ) as a solution of 50 mg/ml in 10-ml vials. All drugs were diluted in sterile 0.9% NaCl.

Drug Doses and Schedules. CPT-11 and FUra were administered by i.v. push once a week for 4 weeks (weekly × 4, on days 0, 7, 14, and 21) at various doses (from a range of 6.25 to 200 mg/kg/week). Combinations of CPT-11 and FUra were administered with the same schedule and route according to three different sequences: (a) CPT-11 and FUra administered as a simultaneous injection (sequence I); (b) FUra 24 h before CPT-11 (sequence II); and (c) CPT-11 24 h before FUra (sequence III).

Received 2/21/00; accepted 5/31/00.

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1 Supported in part by CA65761 and Cancer Center Support Grant CA16056 from the National Cancer Institute, and by Pharmacia & Upjohn Company.

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3 The abbreviations used are: FUra, 5-fluorouracil; CPT, camptothecin; Irinotecan (CPT-11), 7-ethyl-10-[4-(1-piperidino)1-piperidino]carbonyloxycampothecin; CR, complete tumor regression; PR, partial tumor regression; MTD, maximum tolerated dose; MWL, maximum weight loss; TS, thymidine synthase.

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MTD and Toxicity Evaluation. The MTD was defined as the maximum dose that caused no drug-related lethality and which produced animal body weight loss of <20% of original weight. The kinetics of drug-induced toxicities (body weight loss, diarrhea, and lethality) were determined daily for a minimum of 4 weeks and observed at least twice a week thereafter.

Antitumor Activity. Drug treatments were initiated 12–14 days after s.c. tumor transplantation, when tumor weight was \( \sim 3.0 \text{ g} \), as described previously (16). Each group had four rats/experiment, and each experiment was repeated at least three times. Tumor response was expressed as PR when tumor weight was temporarily reduced by at least 50% and as CR when tumor was undetectable by palpation up to 90 days after therapy (cure), at which time the animals were sacrificed. The response rate was expressed as the percentage of animals in the group. All studies were performed in accordance with Institutional Animal Care and Use Committee guidelines and under an approved Institute protocol.

Statistical Analysis. The differences between the mean values were analyzed for significance using the unpaired two-tailed Student’s t test for independent samples; \( P \leq 0.05 \) was considered to be statistically significant.

Results

**Determination of the MTDs of CPT-11 and FUra.** To identify the MTDs of CPT-11 and FUra, Fisher rats with or without Ward colon tumor were treated with various drug doses, administered by i.v. push weekly \( \times 4 \) (on days 0, 7, 14, and 21). Drug-induced deaths were observed at the 150 mg/kg/week CPT-11 dose level and above (25% death at 150 mg/kg and 100% death at 200 mg/kg). The MTD was determined to be 100 mg/kg/week \( \times 4 \) (total dose 400 mg/kg), with no toxicity-related death and MWL ranging from 11 to 19% (mean, 15%).

With FUra, drug-induced deaths were observed at the 125-mg/kg/week dose level and above (25% death at 125 mg/kg, 75% death at 150 mg/kg, and 100% death at 200 mg/kg). The MTD for FUra was therefore also determined to be 100 mg/kg/week \( \times 4 \) (total dose 400 mg/kg), with no toxicity-related death and MWL ranging from 10 to 18% (mean, 14%). In general, because lethality was observed in a significant number of animals when body weight loss exceeded 20% of the initial weight, the MTD was defined as the drug dose that produced no drug-related lethality and \( \leq 20\% \) MWL.

**Antitumor Activity and Toxicity of CPT-11 in Combination with FUra: Role of Drug Sequence.** After determination of the MTDs of CPT-11 and FUra as single agents, studies were initiated to combine the two agents at 50, 75, and 100% of the MTD according to the three different administration sequences: (a) simultaneous administration of CPT-11 and FUra (sequence I); (b) FUra administered 24 h before CPT-11 (sequence II); and (c) CPT-11 administered 24 h before FUra (sequence III). As a single agent, CPT-11 was only slightly active against Ward colon tumor, with no tumor regression observed up to the MTD (Figs. 1 and 2). The tumor was relatively more sensitive to FUra, yielding \( \sim 30\% \) CR at 50% of the MTD with no significant increase in tumor response when increasing the dose up to the MTD (Figs. 1 and 2). All drug combinations were more active than either drug alone, regardless of the sequence of administration; however, greater tumor growth inhibition and CR (cure) rate were apparent with sequence III (Figs. 1 and 2). When combining the two agents at the 50% of MTD, the CR rates were 62, 38, and 95% for schedules I, II, and III, respectively (Fig. 1A). There are statistically significant differences in CR rate between sequence I and III \( (P < 0.05) \) and sequence II and III \( (P < 0.01) \) at 50 mg/kg level (Fig. 1A). There is still a significant difference in CR rate between sequence I and III \( (P < 0.05) \) compared at optimal dose (75 mg/kg with sequence I and 50 mg/kg with sequence III; Fig. 1A and B). For sequences I and II, increasing the drug dose for each drug to 75% of the MTD did not translate into higher antitumor efficacy, nor did it significantly increase toxicity. In contrast, with sequence III, increased toxicity (50–100% lethality) was observed when the FUra dose was increased to 75% of the MTD combined with CPT-11 at 50–75% of the MTD (Fig. 1B). Combining the two drugs at their MTD was highly toxic with all of the three drug sequences evaluated (Fig. 1C).

The kinetics of tumor growth inhibition and toxicity (body weight loss) produced by CPT-11 with or without FUra at 50% of the MTD of each drug are shown in Fig. 2. The data demonstrate the superiority of sequence III over the other two sequences when CPT-11 and FUra doses were kept at 50% of the MTD for each drug.

**Antitumor Activity and Toxicity of CPT-11 in Combination with FUra: Role of Drug Dose.** After identification of the optimal drug administration sequence (sequence III), studies were performed to determine the relationship between drug dose ratios and antitumor efficacy with the CPT-11/FUra combination. A wide range of doses of CPT-11 (from 6.25 to 100 mg/kg/week) and FUra (from 12.5 to 100 mg/kg/week) were used (Fig. 3). Interestingly, CPT-11 at 6.25 mg/kg (62.5% MTD) could still potentiate the antitumor activity of FUra with higher CR rates when FUra was used at 50% of the MTD (Fig. 3).
The data in Table 1 are a summary of the antitumor efficacy of CPT-11 ± FUra with three different administration sequences at various doses of CPT-11 and FUra alone and in combination up to the MTD. There are significant differences in CR rate between sequence I and sequence III (P < 0.05) and sequence II and sequence III (P < 0.01) compared at the optimal therapeutic combination, i.e., 100 mg/kg of CPT-11 and 50 mg/kg of FUra for sequence I, 100 mg/kg of CPT-11 and 50 mg/kg of FUra for sequence II, and 50 mg/kg of CPT-11 and 50 mg/kg of FUra for sequence III. The data provided herein clearly demonstrate the critical role of drug dose and sequence to therapeutic efficacy of the combination of CPT-11 with FUra. Sequential combination of CPT-11 24 h before FUra (sequence III) is the optimal sequence with greatest therapeutic efficacy and selectivity. To achieve high antitumor activity, the doses of FUra should be kept at 50–75% of the MTD, whereas CPT-11 doses can be lowered well-below the MTD (as low as 12.5% of the MTD).

Discussion

Although the combination of CPT-11 with FUra/LV is under clinical evaluation with significant antitumor activity and toxicity (17, 18), the optimal dose-sequence relationship has not been fully defined. Studies herein were performed to determine the role of drug dose and sequence for the CPT-11/FUra combination in a colorectal tumor model, to identify the optimal conditions for achieving maximal synergistic antitumor efficacy and selectivity, and to provide a rationale for clinical development of this combination. Studies by Aschele et al. (19) demonstrated that significant in vitro synergy was achieved when cells were treated with SN-38 first and 24 h later followed by Raltitrexed, a specific TS inhibitor. Saltz et al. (17) demonstrated that a weekly schedule of CPT-11/FUra/LV combination is superior to a daily schedule in patients with previously untreated advanced colorectal cancer. The data demonstrate herein, using a colorectal tumor model with weekly schedule, when compared at the MTD, that all of the drug combinations were more active than each drug by itself, regardless of the drug sequence, but the optimal sequence achieving the highest CR (cure) rate was observed with sequence III (CPT-11 was given 24 h before FUra). Both antitumor activity and toxicity were significantly higher with this sequence compared with the others.

The same sequence-dependent efficacy of CPT-11/FUra combination was also observed in human tumor xenografts of colon cancers HCT-8 and HT-29 tumors and in head and neck cancer A253 and FaDu models. The superiority of antitumor efficacy of CPT-11 sequential combination with FUra (sequence III) is even more significant against these human tumor xenografts. For example, in those treated nude mice bearing HCT-8 colon xenografts with 50 mg/kg of CPT-11 and FUra, 80% of CR (cure) was achieved with combination sequence III, whereas 20, 0, 20, and 10% of CR was achieved with CPT-11 alone, FUra alone, combination sequence I, and combination sequence II, respectively. In addition to the drug administration sequence, the role of drug dose used also proved to be an important factor for the CPT-11/FUra combination. With sequence III, CPT-11 could be used as low as 12.5% of the MTD and still be equally highly curative in the combination as compared with the MTD, whereas FUra had to be used at least at 50% of the MTD to maintain the antitumor activity of the combination. Increasing the dose of FUra to the level of MTD did not translate into additional therapeutic benefit but was associated with increased toxicity (Fig. 3B). The antitumor activity was completely lost when FUra was used at 25 mg/kg (25% MTD) or below (Fig. 3D). This finding is consistent with the data reported by Aschele et al. (19), who demonstrated that the synergy between SN-38 and Raltitrexed was greater when either equiactive doses of the two agents or higher doses of TS inhibitor (Raltitrexed) were used. Thus, in the combination, FUra doses appear to be critical for the antitumor activity, whereas CPT-11 doses appear to play a modulatory role in sensitizing the tumor cells for the subsequent (24-h) FUra treatment.

The mechanism(s) of sequence and dose dependency with CPT-11/FUra combination have yet to be fully defined. Mullany et al. (20) observed similar sequence-dependent effect with SN-38/FUra/LV in HCT-8 cells in vitro. These authors further demonstrated that as a result of SN-38 exposure, the deoxynucleotide dTTP levels were increased and dUTP levels were decreased. The increased dTTP pools may inhibit TS by depletion of dUMP and through this mechanism be (partially) responsible for potentiation of FUra-associated TS inhibi-

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4 S. Cao and Y. M. Rustum, unpublished data.
tion (20). In a study of SN38/FUra on six human colon cancer cell lines by Pavillard et al. (13), they showed the most cytotoxic schedule with was also SN-38 following by FUra. These authors observed an inverse correlation between TS activity and topoisomerase I cleavable complexes (13). Guichard et al. (21) found that CPT-11 treatment decreased TS activity in HT-29 cells, and this decrease persisted for 24 h. However, they observed a strong synergism between CPT-11 and FUra after sequential exposure, regardless of the sequence of drug exposure and additivity or antagonism after simultaneous exposure in vitro (15, 21) and in vivo (15). In the present study, the most active sequence is CPT-11 administered 24 h before FUra (sequence III), and the least active sequence was FUra 24 h before CPT-11 (sequence II, Figs. 1–3). The discrepancy between our results and that of Guichard et al. (21) may be related to a difference in administration schedule. The synergy achieved with the weekly 3 schedule of CPT-11/FUra combination was not achieved in the same model with daily 3 of FUra with CPT-11 as a single dose.

Diarrhea was one of the major dose-limiting toxicities for FUra and CPT-11 in patients (2–8) and animal models (22, 23). Therefore, when combining the two agents, the potential of severe diarrhea may exist. In this study, the incidence of diarrhea was not increased by the CPT-11/FUra combination as long as the doses of CPT-11 and FUra are kept at ≤75% of the MTD.

We also studied the effect of LV modulation with CPT-11/FUra combination with both weekly 4 and daily 5 schedules in the same tumor model. Addition of LV did not increase the antitumor activity of CPT-11/FUra but significantly enhanced the host toxicity (higher body weight loss, diarrhea, and lethality; data not shown).

In summary, although the mechanisms of interaction of CPT-11 and FUra need to be investigated further, drug dose and sequence of administration are critical determinants for therapeutic selectivity and efficacy of CPT-11/FUra combination. The antitumor activity and toxicity of CPT-11/FUra combination are dose and sequence dependent. CPT-11 administered 24 h before FUra (sequence III) is the most active sequence in rats bearing advanced colorectal cancer, and the observed effects in this model system were also confirmed in other tumor models of human colorectal and head and neck cancers, demonstrating the generality of the concept. With sequence III, optimal therapeutic selectivity and antitumor efficacy were achieved when FUra dose was kept at 50–75% of its MTD with CPT-11 at 50% of the MTD.

Table 1 shows the antitumor activity and toxicity of CPT-11 ± FUra in rats bearing advanced colorectal carcinoma: role of drug sequence and dose.

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* mg/kg/week.
* MTD.
* Mean ± SD.
* There are significant differences in CR rate between sequence I and III, II and III compared at the optimal doses (I:III, *P* < 0.05, and II:III, *P* < 0.01). The data are combined from three to five independent experiments, 12–20 rats in total for each experimental group.
MTD or lower. Determination of what FUra doses to be used is critical for successful clinical development of this combination. LV did not further potentiate antitumor activity but significantly enhanced the toxicity of CPT-11/FUra. Because therapeutic efficacy was carried out using clinically active drug combinations and clinically relevant schedules, the therapeutic benefit achieved in the preclinical model system could have significant clinical relevance. The weekly sequential administration of CPT-11 24 h before FUra should be evaluated clinically for its efficacy and selectivity over existing schedules.

References

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Cancer Res 2000;60:3717-3721.

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