Antitumor Efficacy of Edotecarin as a Single Agent and in Combination with Chemotherapy Agents in a Xenograft Model

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Abstract

The novel indolocarbazole edotecarin (J-107088, formerly ED-749) differs from other topoisomerase I inhibitors both pharmacokinetically and pharmacodynamically. In vitro, it is more potent than camptothecins and has a variable cytotoxic activity in 31 different human cancer cell lines. Edotecarin also possesses greater than additive inhibitory effects on cell proliferation when used in combination with other agents tested in vitro against various cancer cell lines. The present in vivo studies were done to extend the in vitro findings to characterize the antitumor effects of edotecarin when used either alone or in combination with other agents (i.e., 5-fluorouracil, irinotecan, cisplatin, oxaliplatin, and SU11248) in the HCT-116 human colon cancer xenograft model. Treatment effects were based on the delay in onset of an exponential growth of tumors in drug-treated versus vehicle control-treated groups. In all studies, edotecarin was active both as a single agent and in combination with other agents. Combination therapy resulted in greater than additive effects, the extent of which depended on the specific dosage regimen. Toxicity in these experiments was minimal. Of all 359 treated mice, the six that died of toxicity were in the high-dose edotecarin/oxaliplatin group. The results suggest that edotecarin may serve as effective chemotherapy of colon cancer when used as a single agent, in combination with standard regimens and other topoisomerase inhibitors or with novel agents, such as the multitargeted tyrosine kinase inhibitor SU11248.

Colorectal cancer remains a major cause of cancer-related mortality for both men and women and, up until the year 2000, patients with metastatic disease survived an average of 12 months (1). Until that time, 5-fluorouracil (5-FU) administered either with or without leucovorin had been the standard chemotherapy agent used for human colon cancer since its initial clinical evaluation (1). In recent years, outcomes have been further improved by the addition of the topoisomerase I inhibitor irinotecan or the platinum compound oxaliplatin, which are now considered standard agents to use in conjunction with 5-FU for managing recurrent or advanced colorectal cancer (2–5). The combination of irinotecan and oxaliplatin has also been tested but has not been shown to be superior to other regimens (6). Currently, 5-FU/irinotecan is emerging as an important combination partner for additional targeted agents, which significantly extend survival in patients with metastatic disease. For instance, targeted monoclonal antibody–based agents directed against vascular endothelial growth factor (e.g., bevacizumab) or epidermal growth factor receptor (e.g., cetuximab) have recently received Food and Drug Administration approval in 5-FU/irinotecan–based chemotherapy regimens (7–9).

Edotecarin (J-107088, formerly ED-749) is a potent and specific indolocarbazole inhibitor of topoisomerase I derived from NB 506 (10). Its chemical structure differs from camptothecin-derived, topoisomerase I inhibitors that are currently in clinical use or undergoing investigation (10–14). Like other topoisomerase I inhibitors, edotecarin prevents the relaxation of torsionally strained duplex DNA essential for DNA replication and transcription, but differs in its pharmacokinetic (15) and pharmacodynamic (10, 16) profiles. The topoisomerase cleavage complexes induced by edotecarin are more stable than those induced by either its parent compound or camptothecin, as measured both by in vitro DNA cleavage assays and in DNA cleavage assays done using cultured cells (10, 11). On the basis of its ability to inhibit topoisomerase-mediated DNA cleavage in vitro, edotecarin is 8-fold more potent than camptothecin, with an effective concentration value ($EC_{50}$) of 0.05 μmol/L compared with 0.42 μmol/L for camptothecin (10).

The antitumor efficacy of edotecarin, when used as a single agent, has been characterized in vitro with human cancer cells and in vivo with human tumor models in nude mice (10, 15, 17). For example, cytotoxic activity against a panel of 31 human cancer cells has been shown, with $IC_{50}$ values ranging from 0.0015 to 0.39 μmol/L (10, 15). In addition, the spectrum of activity of edotecarin against human cancer cell lines was found to differ from camptothecin, doxurubicin, etoposide, and cisplatin (10). In several different human tumor...
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Materials and Methods

Animals. Athymic male BALB nu/nu mice were used in all studies. The mice were housed under pathogen-free conditions in microisolator cages, with irradiated rodent chow and water available ad libitum. These animal studies were done using procedures in compliance with Italian Legislative Decree no.116, January 27, 1992, enforcing the European Communities Council Directive N.86/609/EEC concerning the protection of animals used for experimental or other scientific purposes, and its effects in combination with other active chemotherapeutic agents would be additive or perhaps synergistic.

The present studies were done to examine the antitumor activity of edotecarin when used as a single agent or in combination with other agents in vivo on the HCT-116 human colon cancer xenograft model. Specifically, the antitumor activity of edotecarin was examined in combination with either one of the standard chemotherapy drugs (i.e., 5-FU, irinotecan, or oxaliplatin) or with SU11248, a novel multitargeted, tyrosine kinase inhibitor (23–25). Edotecarin was found to have potent antitumor activity alone and to have additive or more than additive activities in combination with other active agents in this model system for human colon cancer.

Test compounds. All compounds were prepared immediately before use. Drug doses and treatment schedules were mainly based on data from separate unpublished internal studies, as well as from the literature. As a general principle, 70% of the maximum tolerated dose of the second drug was combined with edotecarin. The 5-FU dose was 50 mg/kg based on maximum tolerated dose reported by Houghton et al. (20), the irinotecan dose was 45 mg/kg as described previously (26), the cisplatin dose of 5 mg/kg was estimated from Keane et al. (21), the oxaliplatin dose was 10 mg/kg as described previously (27, 28), and the SU-11248 doses of 20 and 40 mg/kg were based on data from preclinical studies (23–25). Edotecarin was dissolved in 20% polyethylene glycol 400 and water. The administration schedule for edotecarin—once or twice weekly for ≥2 weeks—was based on unpublished data showing the equivalence of the two schedules (data not shown): The two regimens allowed adaptation of the edotecarin schedule to that of the best schedule of the second drug. Cisplatin and 5-FU were dissolved in water, irinotecan, and oxaliplatin in glucosate water, and SU11248 in Methocel cellulose ethers (The Dow Chemical Company, Midland, MI). Unless noted, all agents were administered i.v. in a volume of 10 mL/kg body weight, and eight mice per group were treated.

Evaluation of antitumor activity. Tumor growth and net body weight were evaluated every 3 days. Tumor growth was assessed by using calipers. Tumor weight was calculated according to the following formula: length (mm) × width² (mm) / 2. The effect of treatment was determined as the delay in onset of an exponential growth of tumors (29). The delay was expressed as the T-C value, which was defined as the difference in median times (in days) required for the treatment and control group tumors to reach the predetermined size of 1 g. Toxicity was evaluated based on body weight reduction. Mice were sacrificed when tumors reached a volume that hampered them. Gross autopsy findings were noted and reported (primarily reduction in spleen and liver size). Animals that were tumor-free at 90 days after tumor implant were considered cured.

Statistical analyses. The Mann-Whitney U test was used to test differences between treatment groups. This analysis was done for every evaluation of tumor weight and at the day of maximum net body weight loss using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA). P < 0.05 was considered statistically significant.

In vitro studies. HCT-116 human colon carcinoma cells were grown in McCoy’s medium supplemented with 10% FCS. Exponentially growing HCT-116 cells were seeded at 5,000/cm² in 96-well plates (Black ViewPlate-96; Packard Bioscience, Meriden, CT) and incubated at 37°C in a humidified 5% CO₂ atmosphere. After 24 hours, scalar doses of test compounds were added to the medium, and cells were treated for 72 hours in contemporaneous experiments or for 24 hours with the first drug followed by a treatment for 48 hours with the second drug in sequential experiments. At the end of treatment, cell proliferation was determined by a cellular ATP monitoring system (Promega Corporation, Madison, WI), which consists of a mixture of luciferase and luciferin reconstituted in a lysis buffer; 100 µl/well of this solution were added to the cells. The plates were then shaken for 2 minutes in an orbital shaker and were read in a luminometer, with the signal linearly proportional to the cell number. Calculations of combination indexes were done using the CalcuSyn program (Biosoft, Cambridge, United Kingdom).

Results

Edotecarin in combination with 5-FU. The antitumor efficacy of edotecarin alone and in combination with 5-FU was assessed by using calipers every 3 days. Tumor growth was assessed by using calipers every 3 days.

Fig. 1. Comparison of tumor growth in control and edotecarin-, 5-FU-, and edotecarin/5-FU–treated groups. Edotecarin was administered as a single agent in one of four doses or 1 hour before 5-FU (combination groups). Tumors were measured by using calipers every 3 days. Treatments were administered weekly for 4 weeks. Tumor growth was assessed by using calipers every 3 days.

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xenograft models in nude mice, and using a broad range of malignancies, edotecarin when used as a single agent produced significant antitumor activity (15, 17, 18). Xenograft models have also been useful in preclinical systems to test the antitumor activity of the topoisomerase I inhibitors irinotecan and topotecan when used in combination with either cisplatin or 5-FU. These combinations were active against several tumors, including colon adenocarcinoma (19–22). Based on these preclinical data, it was hypothesized that edotecarin would be active as a single agent against colon adenocarcinoma and that its effects in combination with other active chemotherapeutic agents would be additive or perhaps synergistic.

Tumorigrowthwasassessedbyusingcalipersevery3days.

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5-FU-,andedotecarin/5-FU^treatedgroups.Edotecarinwas

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examined. Irinotecan administered as a single agent was used as the reference compound. The objective of this study was to determine if the sequence of administration influenced the antitumor activity of edotecarin in combination with 5-FU, both in vitro and in vivo, on the HCT-116 model. As a single agent, edotecarin resulted in tumor growth delays ranging from 10.45 days at the lowest dose (3 mg/kg) to 24.83 days at the highest (100 mg/kg). When edotecarin was given 1 hour after 5-FU, only the lowest dose of edotecarin (3 mg/kg) used in combination showed antitumor efficacy superior to edotecarin alone: $P \leq 0.01$ from day 22 (Fig. 1; Supplementary Table S1A).

In the sequencing studies, the results of in vitro experiments did not reveal significant differences between the two regimens; that is, 5-FU followed by edotecarin versus edotecarin followed by 5-FU. For both treatment sequences, the combination indices were calculated using a computer program for multiple drug effect analysis based on the equation of Chou-Talalay (30) for mutually nonexclusive drugs. The means of the combination indices (calculated when the fraction affected was $>0.5$) were $0.85 \pm 0.21$ for edotecarin followed by 5-FU and $0.63 \pm 0.19$ for the opposite sequence, indicating additive or greater than additive activity.

Sequencing of the administration of 5-FU and edotecarin with a 24-hour interval did not improve efficacy of the combination over edotecarin alone, regardless of the agent administered first (Fig. 2; Supplementary Table S2A). A 72-hour interval between agents, however, seemed to provide greater antitumor efficacy than did edotecarin given as a single agent ($P < 0.01$ from day 44). The tumor growth delay was $49.99 \pm 5.17$ days in the group receiving the combination with a 72-hour interval versus $42.45 \pm 2.48$ days in the group receiving edotecarin as a single agent ($P < 0.005$).

**Edotecarin in combination with irinotecan.** The combination of two topoisomerase I inhibitors was supported by their mode of action partially different, as reported by Yoshinari et al. (10), and by the observation that camptothecin-resistant cell lines (L1210/CPT and L1210/9AC) were sensitive to edotecarin (Supplementary Table S3A). The results of in vitro experiments with SN-38 (the active metabolite of irinotecan) revealed greater than additive activity; in fact, the combination index was $0.55 \pm 0.22$ [calculated when the fraction affected was $>0.5$ by the equation of Chou-Talalay (30) for mutually nonexclusive drugs]. Combination treatment of edotecarin plus irinotecan improved antitumor activity in vivo compared with either agent alone (Fig. 3; Supplementary Table S4A). For all combinations, the tumor growth delay value was significantly higher than that for the corresponding doses of edotecarin or irinotecan alone ($P < 0.002$). One subject in the group with 30 mg/kg edotecarin plus 45 mg/kg irinotecan was rendered tumor-free. Tumor growth was consistently slower in each combination treatment group than in the single-agent groups ($P < 0.05$ from day 41).

**Edotecarin in combination with cisplatin.** All combinations of edotecarin with cisplatin resulted in a significantly greater than additive antitumor effect on all variables measured: $P < 0.01$ for 3 mg/kg from day 19, 10 mg/kg at day 33 only, and 30 mg/kg from day 26 (Fig. 4; Supplementary Table S5A).

**Edotecarin in combination with oxaliplatin.** The combination of 10 mg/kg oxaliplatin with 3 mg/kg edotecarin resulted in a greater than additive antitumor effect (Fig. 5; Supplementary Table S6A): $P < 0.005$ from day 36, when the tumor growth curve of the combination was compared with that of 3 mg/kg edotecarin alone. The tumor growth delay for the combination of 3 mg/kg edotecarin plus oxaliplatin was 16.2
versus 1 day for oxaliplatin alone and 8.2 days for 3 mg/kg edotecarin alone. Although a greater tumor growth delay was observed with the higher-dose edotecarin (30 mg/kg) plus oxaliplatin combination, this combination was also associated with increased toxicity, resulting in the death of six of eight mice in the group.

**Edotecarin in combination with SU11248.** All combinations of SU11248 with either edotecarin or irinotecan produced significantly greater than additive antitumor responses (Fig. 6; Supplementary Table S7A). Further, three of five evaluable mice were rendered tumor-free by the end of treatment with the combination of SU11248 at 40 mg/kg plus edotecarin; however, when treated with edotecarin alone, the tumor regrew at the end of treatment in all treated mice.

**Toxicity.** The results across all experiments confirmed the previously reported high margin of safety observed with edotecarin (17). In general, decreases in net body weight were comparable across treatments within a study and did not seem to differ significantly (Supplementary Table S8A). Statistically significant differences between groups (P < 0.01) were observed only in the groups treated with cisplatin both alone and in combination with all three doses of edotecarin, and in the group treated with 60 mg/kg irinotecan. Moreover, body weight decreases in groups receiving combination treatments did not exceed the sum of the decreases associated with each of the single agents. Of the 45 treatment groups with a total of 359 mice included in these studies, deaths due to toxicity were noted in only one treatment group: six of eight mice treated with 10 mg/kg oxaliplatin plus 30 mg/kg edotecarin.

**Discussion**

The initial exploration of topoisomerase inhibitors for the treatment of colon cancer was based on finding that concentrations of this enzyme were significantly increased in malignant compared with normal colonic tissue from advanced human colon adenocarcinoma and from xenografts of colon cancer from immunodeficient mice (31). The topoisomerase inhibitor irinotecan—developed to overcome some of the disadvantages of the natural camptothecin compound—is a component of several of the chemotherapeutic regimens used in the treatment of colon cancer. Although irinotecan-containing regimens have improved outcomes, the prognosis remains poor for metastatic disease, thus underscoring the need for trials with other agents. The preclinical profile of the novel indolocarbazole topoisomerase I inhibitor edotecarin has suggested that this new agent may offer clinical advantages over existing camptothecin-derived topoisomerase inhibitors. Specifically, edotecarin is more potent than camptothecin by an 8-fold margin and has a longer duration of action, as evidenced by the stability of DNA-protein complexes (10).

Edotecarin has also been associated with a wider therapeutic window compared with doxorubicin, paclitaxel, and cisplatin based on the ratio of the dose that was lethal to 10% of mice relative to the dose inhibiting the volume of solid tumors by 75% (LD10/GID75; ref. 17). An LD10/GID75 value <1.0 indicates severe toxicity associated with the inhibition of tumor growth. For example, in mice with xenografts of PC-3 human prostate tumor, edotecarin had an LD10/GID75 of 38.5
versus <0.3, <0.5, and <0.2 for doxorubicin, paclitaxel, and cisplatin, respectively (17).

In the present study, using the HCT-116 human colon cancer xenograft model, edotecarin was active as a single agent and in combination with other agents, resulting in greater than additive effects, depending on the dosing and schedule of administration. The doses and administration schedules for the other agents selected in this study were based on published literature indicating them to be efficacious, but <70% of the maximum tolerated dose for each individual drug was used in the various combination regimens.

The findings of these in vitro experiments using the HCT-116 human colon cancer xenograft model confirm the potential use of edotecarin to treat human colon cancer and indicate that the advantages exhibited by edotecarin in vitro may translate to improved clinical benefits without increased toxicity. Specifically, the activity of edotecarin with 5-FU suggests that edotecarin may be useful for first- and second-line regimens that incorporate a topoisomerase I inhibitor in combination with 5-FU/leucovorin. The order of administration of the two agents revealed no effect of sequencing either in vitro or in vivo. The longer 72-hour interval between 5-FU and edotecarin, however, was more efficacious than the 24-hour interval, which may have implications for clinical testing; however, sequencing of edotecarin before 5-FU was not evaluated in that experiment.

Edotecarin administration also improved the antitumor activity observed with another topoisomerase I inhibitor—irinotecan—as well as with oxaliplatin, cisplatin, and the oral, multitargeted, tyrosine kinase inhibitor SU11248. In all mice treated with these combinations, the delay in tumor growth was significantly longer than in those treated with edotecarin alone or did not grow at all, as in the case of one of eight mice treated with the combination edotecarin and irinotecan, and three of five mice treated with the combination of edotecarin and SU11248. With a single exception, that is, high-dose edotecarin plus oxaliplatin, toxicity was not increased by combining edotecarin with other agents, confirming the wide margin of safety previously reported (17).

Irinotecan and oxaliplatin are currently used as front-line agents of choice in the treatment of metastatic colorectal cancer in humans (2–6). SU11248 has antitumor and antiangiogenic activities that result from multitargeting platelet-derived growth factor receptor, vascular endothelial growth factor receptor, KIT receptor tyrosine kinases, and fms-related tyrosine kinase 3/Flik 2 (23–25). This new agent inhibits ligand-dependent receptor phosphorylation and cell proliferation, and its activity is at least additive when used with cytotoxic agents. Antiangiogenic therapy has been recognized as an important new component to combination chemotherapy for metastatic colon cancer with the advent of targeted therapeutic agents such as bevacizumab (8, 9). Taken together, these results suggest that edotecarin may be a potent and well-tolerated agent for use in future combination chemotherapy regimens for human colon cancer.

References


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