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Randomized clinical trial of mitomycin-C with or without pretreatment with WR-2721 in patients with advanced colorectal cancer

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Abstract. The use of mitomycin for metastatic colorectal cancer has been limited by mitomycin's myelosuppressive potential. The objective of this randomized study was to determine whether WR-2721 would decrease the hematologic toxicity of mitomycin in patients with colorectal cancer resistant to fluorouracil-based therapy. Ninety-seven patients with refractory colorectal cancer were randomized to receive either mitomycin 20 mg/m^2 only or the same dose of mitomycin after pretreatment with WR-2721, 910 mg/m^2. The principal toxicity in both groups was thrombocytopenia. The platelet nadirs were lower in patients receiving single-agent mitomycin ($P = 0.026$). Surprisingly, no clinical complete or partial responses were noted in either group, and survival was not different between the two groups. Thus, while WR-2721 decreased the thrombocytopenia associated with mitomycin therapy, mitomycin was ineffective in the treatment of refractory colorectal carcinoma.

Introduction

Mitomycin has been utilized for the treatment of colon and rectal adenocarcinoma since 1959 [1, 2, 6, 10, 11]. Multiple studies have reported a clinical response frequency ranging from zero to a high of 33% [1, 2, 6, 10, 11]. Unfortunately, treatment utilizing mitomycin is complicated by often severe, cumulative and prolonged myelosuppression as well as infrequent occurrences of hemolytic-uremic syndrome and pulmonary insufficiency, which require the cessation of therapy despite tumor response.

WR-2721 (*S*-2-[3-aminopropylamine] ethyl phosphorothioic acid) is an organic thiophosphate compound that, in animal models, protects against the cytotoxicity of cisplatin and alkylating agent chemotherapy (U. S. Bioscience file data, unpublished). Circulating WR-2721 is converted by capillary alkaline phosphatase to an active moiety, WR-1065. While WR-1065 is absorbed rapidly by normal cells, malignant cells absorb only small amounts of the drug. The higher pH and activity of alkaline phosphatase in normal tissues than in tumors contribute to the differential absorption and benefit of WR-2721. In the animal model, WR-2721 attenuates the toxicities of cyclophosphamide, nitrogen mustard and melphalan as well as cisplatin. The pretreatment of mice and rats with WR-2721 produces a 1.7- to 2.5-fold increase in resistance both to hematopoietic injury from alkylators and to nephrotoxicity induced by cisplatin, while maintaining the antineoplastic effect of the compounds. Preliminary murine data have demonstrated that WR-2721 (400 mg/kg) can decrease the myelosuppressive effect of mitomycin (4.5 mg/kg, the LD_{10} dose) without loss of the antitumor effect (U. S. Bioscience file data, unpublished).

It was postulated, based on this preclinical data, that the addition of WR-2721 to therapy with mitomycin in the treatment of patients with colorectal cancer would limit myelosuppression, thus allowing full-dose mitomycin treatment to continue as long as the patient continued to respond. To test this hypothesis, a multi-institution randomized trial was undertaken with a dose level of mitomycin 20 mg/m^2, with or without pretreatment with WR-2721, in patients with previously treated colorectal cancer.

Patients and methods

All patients with colorectal adenocarcinoma who had progressive disease after treatment with a fluorouracil-containing regimen were considered for treatment. Inclusion required that no chemotherapy had been administered in the previous 2 weeks and no radiation therapy in the prior 12 months. Patients had to have recovered from any toxicity of prior treatment. Patients had to have a performance status of 0–2 (ECOG), measurable disease and adequate marrow, renal and hepatic function as confirmed by WBC >4000 cells/mm^3, platelets >125,000/mm^3, serum creatinine <1.5 mg/dl, and total serum bilirubin <4.0 mg/

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dl. Patients were required to givent written informed informed consent. A patient was excluded if he/she did not meet the eligibility criteria, had received any chemotherapy other than fluorouracil or fluorouracil/leucovorin, or had any prior malignancy (excluding basal or squamous cell cancer of the skin or surgically treated stage I carcinoma of the cervix). Pregnant patients were excluded. Patients with angina pectoris and those receiving antihypertensive medications that could not be safely discontinued for 24 h were also excluded. Patients with active infections were excluded.

WR-2721 was supplied by U. S. Bioscience in vials containing 500 mg of WR-2721 in lyophilized form and 500 mg of mannitol. Each vial of WR-2721 was reconstituted with 9.3 ml of normal saline within 4 h of administration to yield a final solution containing 50 mg/ml of WR-2721. The dose of WR-2721 to be administered was 910 mg/m^2. Mitomycin-C was obtained commercially and was reconstituted with sterile water according to the manufacturer's instructions. The dose of mitomycin to be administered was 20 mg/m^2.

Patients were randomized to receive either mitomycin 20 mg/m^2 only or mitomycin 20 mg/m^2 plus WR-2721, 910 mg/m^2, delivered 15 min before mitomycin. The treatment was repeated every 6 weeks. Patients who were to receive the combination with WR-2721 were prehydrated before each treatment. WR-2721 was administered over 15 min to patients maintained in the supine position. The patient's blood pressure was monitored every 5 min during the infusion and for 5 min after the completion of the infusion. The infusion was interrupted if the systolic blood pressure dropped by more than 20%. Therapy could be reinstituted only if the blood pressure normalized within the subsequent 5 min. The dose of WR-2721 was reduced for subsequent cycles in patients whose WR-2721 had to be terminated prematurely because of hypotension. Mitomycin 20 mg/m^2 was administered by i.v. bolus 5 min after completion of the WR-2721 infusion. Patients who were randomized to receive mitomycin 20 mg/m^2 only received their drug by i.v. infusion with no mandated prehydration or hospitalization. Antiemetics were limited to lorazepam, metoclopramide and diphenhydramine.

Subsequent courses of mitomycin were given in reduced doses if significant thrombocytopenia or neutropenia or decreased renal function was noted. Further treatment was withheld if the WBC remained below 1500 cells/mm^3 or the platelet count remained below 100,000/mm^3 for more than 2 weeks after the next scheduled treatment. Therapy was also halted if the creatinine rose to over 2.0 mg/dl or if any evidence of hemolytic-uremic syndrome or pulmonary toxicity developed.

Patients were evaluable for response and toxicity if they had received one course of therapy.

Tumor response was based upon follow-up observation of the indicator lesions noted when the patient was registered. A complete response was defined as the disappearance of all indicator lesions, documented by two observations at least 4 weeks apart, with no new lesions appearing and with normalization of any previously abnormal markers. A partial response was defined as a decrease of over 50% in the sum of the products of all bidimensionally measured tumor masses, documented by two observations at least 4 weeks apart with no new lesions appearing or progression of any existing lesions. Progression was defined as over 25% increase in the sum of the products of all bidimensionally measured tumor masses, the appearance of any new lesion, recurrent disease if the patient had achieved a complete response, or significant deterioration in performance status thought to be related to the underlying cancer. All patients who had been on study for at least 4 weeks and did not meet the criteria for response or progression were considered to have stable disease.

Patients were removed from the trial if significant hematologic, pulmonary, renal toxicity was observed or any grade IV toxicity was observed. Toxicity was graded using the WHO criteria. Patients were similarly removed from protocol treatment if there was evidence of disease progression. Patients were also removed from study if they so requested. Follow-up studies were obtained on all patients until their deaths.

Tests of significance were performed using Pearson's Chi-square test for 2×2 contingency tables and the Mantel-Haenzel Chi-square test for contingency tables larger than 2×2. Differences in mean nadir counts were also compared between the two treatment groups using T-tests. Two-sided tests of significance are reported. Survival and time to progression are calculated from the first day of therapy. Survival is the time to death. The survival and time-to-progression curves and medians are estimated using the Kaplan-Meier (or product limit) method. The hazard ratios and corresponding 0.95 confidence intervals comparing the curves and tests of significance were performed using the log-rank test, which weights the times equally, and the Wilcoxon test, which weights each time with a function of the number of patients at risk at the time of the event and places greater weights on early events. Two-sided tests of significance are reported.

Results

In all, 97 patients from 24 participating institutions were eligible and received therapy from 7 September 1988 through 30 August 1990. Forty-eight were randomized to receive mitomycin and WR-2721 and 49 to mitomycin alone. One additional patient randomized to receive the combination was deemed ineligible after randomization and never received any treatment on protocol. The baseline characteristics of the groups do not differ significantly (Table 1). There were more men receiving mitomycin alone than the combination. In both groups, the vast majority of patients had good to excellent performance status (ECOG 0–1). The majority of patients were also reported to have

Table 1. Baseline characteristics

Parameter	WR + Mitomycin (n = 48)		Mitomycin (n = 49)	
	n	%	n	%
Age (years) median (range)	61	(34–77)	60	(39–82)
Sex				
Male	25	52.1	33	67.4
Female	23	47.9	16	32.6
ECOG Performance status				
0 – Fully active	18	37.5	25	51.0
1 – Ambulatory	26	54.2	20	40.8
2 – Bedridden <50%	4	8.3	4	8.2
Previous therapy				
5-FU	38	79.2	30	61.2
5-FU + leucovorin	10	20.8	19	38.8
Response to prior 5-FU therapy				
Response followed by progression	32	66.7	37	75.5
No response followed by progression	16	33.3	12	24.5
Duration of response to prior 5-FU therapy (months) median (range)	6.0	(0.7–59.9)	4.6	(0.3–33.6)
Prior radiotherapy				
Yes	9	18.8	6	12.2
No	39	81.2	43	87.8
Residual disease				
<2 cm	4	8.3	5	10.2
≥2 cm to <5 cm	25	52.1	14	28.6
≥5 cm to <10 cm	15	31.2	24	49.0
≥10 cm	4	8.3	6	12.2

Table 2. Myelosuppression: mean nadirs and SEM for granulocyte count, platelet count and hemoglobin

Parameter	Cycle	WR+MMC		MMC		2-sided P-value
		Mean	SEM	Mean	SEM	
Granulocyte count (cells/mm^2)	1	2575	291	2225	198	0.32
	Overall	2236	279	1935	181	0.37
Platelet count (,000/mm^3)	1	159	14	120	11	0.03
	Overall	121	13	94	9	0.09
Hemoglobin (g/dl)	1	10.9	0.3	10.9	0.2	0.91
	Overall	9.9	0.3	10.1	0.2	0.52

responded previously to a fluorouracil-containing regimen, though the median response duration was slightly longer among patients treated with mitomycin and WR-2721.

A total of 97 courses of therapy were delivered in the combination arm and 95 in the single-agent arm. Thirty-three patients were able to receive a second course of mitomycin and 32, a second course of mitomycin and WR-2721. However, there was a much larger decrease in the number of patients receiving further therapy thereafter. Only 15 patients received a third and only 2 a fourth course of the combination treatment. Similarly, only 11 patients received a third cycle and only 2 a fourth cycle of mitomycin alone. Therapy was halted because of disease progression in 31 and 41 patients receiving the WR-2721 and mitomycin combination and mitomycin alone, respectively. Therapy was halted in 9 patients in the combination arm and 5 patients in the single-agent arm because of toxicity. Treatment was halted in 6 patients, all receiving the combination, because of non-compliance, patient request and, in 2 patients, worsening underlying non-oncologic conditions. One of these 2 patients had worsening asbestosis requiring steroids. The other suffered a fatal myocardial infarction 10 days after initiation of therapy. An additional patient, receiving mitomycin alone, succumbed to a sepsis secondary to an underlying perirectal abscess on day 17 of treatment.

No objective tumor responses were noted in either treatment group. The time to progression was 3.0 months for those patients receiving a combination of mitomycin and WR-2721, as against 2.5 months for patients receiving mitomycin alone ($P = 0.018$, log-rank; $P = 0.0496$, Wilcoxon). The median follow-up time for all patients is 13.3 months. The median survival time for patients treated with mitomycin and WR-2721 was 7.2 months (range 0.3–15.1) months, as opposed to 6.3 months (0.6–16.9) for mitomycin alone (not significant).

Hematopoietic toxicity, principally thrombocytopenia, was notable in both treatment arms (Table 2). The severity of thrombocytopenia was attenuated by WR-2721. The mean first course platelet nadirs for the combination and single arms were 159,000/mm^3 and 120,000/mm^3, respectively ($P = 0.03$). The percent decrease from baseline platelet count to the nadir in the first course was 52% for the combination and 60% for mitomycin alone ($P = 0.049$). The incidence of first-cycle grade 3 or 4 thrombocytopenia was 23% in the mitomycin arm and 10% in the combination arm ($P = 0.100$). Grade IV thrombocytopenia (platelets <25,000) was seen in 1 patient receiving his third course of single-agent mitomycin. Five patients receiving the combination developed grade IV thrombocytopenia, 1 in course one, and 2 each in courses two and three. There were no significant differences in hemoglobin levels between the two treatment arms.

The mean first-course granulocyte nadirs in the combination and single-agent arms were 2575/mm^3 and 2225/mm^3, respectively ($P = 0.32$). Four patients in the combination arm and three patients in the mitomycin-alone arm had granulocyte counts <500/mm^3. Unfortunately, cumulative hematopoietic toxicity in each arm cannot be assessed, because 67% of patients received no more than two courses of treatment.

A variety of other toxicities were noted which appeared to be related to mitomycin C. These toxicities did not appear to differ in frequency between the combination and single-agent trial arms. There were five episodes of respiratory distress, which were thought to be possibly or probably the results of mitomycin pulmonary toxicity. These episodes were documented by clinical examination, deterioration in carbon monoxide diffusing capacity and/or by bronchoscopic biopsy. There were 4 occurrences with the combination treatment and 1 with single-agent mitomycin. The patient's symptoms responded to steroids and the discontinuation of chemotherapy treatment. One patient developed hemolytic-uremic syndrome, with documented hemolysis, renal failure and thrombocytopenia, after his third course of single-agent mitomycin. The patient required plasmapheresis as well as subsequent dialysis.

The WR-2721 infusion was discontinued during 13/97 (13.4%) of the cycles among 12 of 48 (25%) patients because of a decrease in the systolic blood pressure. None of the patients had any neurologic or cardiovascular sequelae related to this transient hypotension, and their baseline blood pressure was restored within 10 min of cessation of the WR-2721. For these cycles a median of 88.5% of the drug, 805 mg/m^2, had been delivered. In an additional nine cycles, WR-2721 was interrupted for transient hypotension but was subsequently completed. Patients who developed hypotension received reduced doses of WR-2721 during subsequent cycles.

Only 8 of 49 (15%) patients treated with mitomycin alone experienced vomiting, as against 35 of 48 (73%) patients treated with the combination. Severe vomiting was seen only with the combination and only in three cycles of treatment. Antiemetics were utilized as needed.

A variety of other mild WR-2721 specific side effects were noted: flushing and feelings of warmth (20% of cycles); sneezing (22%); dizziness and lightheadedness (9%); sleepiness and somnolence (4%) hiccups (2%) and chills (2%).

Discussion

In this randomized trial of patients with refractory metastatic colorectal cancer, the utility of WR-2721 in modifying the hematologic toxicity of mitomycin was evaluated. WR-2721 itself engendered modest nausea, vomiting, and occasional hypotension Thrombocytopenia was decreased modestly in patients pretreated with WR-2721. There was no overall difference in survival time between the two patient groups. Time to progression was longer, though minimally, for patients receiving mitomycin and WR-2721 than for those patients receiving mitomycin alone, which is consistent with but does not prove the hypothesis that WR-2721 is not tumor-protective.

Mitomycin had been considered a relatively active agent for the treatment of colon cancer. The absence of responses in this trial was therefore unanticipated, given the multiple trials done 1968–76 demonstrating efficacy in previously treated and untreated patients. In retrospect, the only trial showing no activity was that of Ansfield [1]. In that study, 23 patients, all previously treated with fluoropyrimidines, were treated with mitomycin 0.05 mg/kg per day for six daily doses. There were no responses, though the criteria of response were not stipulated.

The decrease in reported antitumor activity is not unique to mitomycin, however. Fluorouracil as given by 5-day infusion for colorectal cancer had a reported response rate of 44% in 1975 [7] and 3% in 1990 [7]. Similarly, the response rate to the original schedule of 5-FU given by 5-day i.v. bolus was 22% [12], and a more recent report cites 7% [9].

Over the last two decades, both response criteria and methods for assaying response have changed. For Godfrey et al. clinical responses included a "50% decrease in the mass of the measurable lesion... opening of a gastrointestinal obstruction and disappearance of jaundice or pleural effusion" [6]. In the study of Moore et al. a partial response required reduction in one or more lesions by more than 50% for two successive measurements [11]. The WHO criteria, used in this current study, require, for a partial response, 50% reduction in the sum of the products of all bidimensionally measured lesions, no progression of evaluable disease and no development of new lesions, all this to be maintained for at least 4 weeks.

The availability of CT technology for evaluation of intraabdominal disease has obviously changed the evaluability of response. In previous studies where responses were described, the measurable lesions were liver size, abdominal mass size and lymph node size. Ansfield specified the use of lesions seen on chest X-ray or measurable superficial lesions as the only means of response assessment. In contrast, in this study, CT scan assessment of liver abnormalities was used for measurement in 72% of patients, chest X-rays in 10% and abnormalities seen on physical examination in 2%.

WR-2721 has been used in conjunction with other chemotherapeutic agents, in particular cisplatin. In the original studies, there was evidence of protection by WR-2721 from cisplatin-induced nephrotoxicity, myelosuppression and neurotoxicity [10]. Additional data demonstrated that WR-2721 could decrease the severity of granulocytopenia induced by cyclophosphamide [4]. In a recent randomized trial of 121 women with ovarian cancer [3] a treatment regimen of cisplatin 100 mg/m^2 and cyclophosphamide 1000 mg/m^2 was used, with or without pretreatment WR-2721. First-course granulocytopenia was seen in 57% of those treated with WR-2721 and chemotherapy and in 79% of those receiving chemotherapy alone ($P = 0.011$), and thrombocytopenia <50,000 was seen in 0% of those receiving WR-2721, as opposed to 7% in those receiving chemotherapy alone ($P = 0.047$). Persistent nephrotoxicity was rare. Serum creatinine in excess of 1.5 mg/dl was seen in 3% of patients treated with the WR-2721 combination, but in 15% in those treated with chemotherapy alone ($P = 0.04$). After six cycles of therapy, peripheral neurotoxicity more severe than grade 2 occurred in 19% of patients treated with cisplatin, cyclophosphamide and WR-2721, as against 32% treated with chemotherapy alone ($P = 0.022$). Demonstrable complete responses were recorded in 36% of the patients treated with the combination and 29% of those treated with the chemotherapy alone (not significant), indicating preservation of the antitumor response in patients treated with WR-2721.

However, WR-2721 was found less useful in a nonrandomized study of patients with head and neck cancer [8]. A series of 25 patients was treated with a 4-day infusion of fluorouracil 1000 mg/m^2 per day and cisplatin 120 mg/m^2 on the initial day. WR-2721 was given in doses of 740 mg/m^2 and 910 mg/m^2 prior to cisplatin. Patients had a median performance status of 1 and the majority, significant ethanol exposure. Among the 15 evaluable patients, 8 responded. Myelosuppression was modest. However, 20% of patients developed severe ototoxicity and 20% developed serum creatinine levels of 2.8–11.3 mg/dl requiring cessation of therapy. Patients who developed renal dysfunction had either hypertension or a history of moonshine ingestion, which could have predisposed them to more severe toxicity. However, in this group of head and neck cancer patients, treated at a higher dose of cisplatin, significant toxicity was still engendered despite apparent preservation of antitumor activity.

The results of a two-arm group-wide melanoma trial comparing cisplatin 120 mg/m^2 alone with WR-2721 and cisplatin 150 mg/m^2 are awaited. Meanwhile, given the inherent mild to modest toxicity of WR-2721, its use is only indicated for those tumor types and chemotherapy agents where benefit is sufficient to make the additional toxicity generated by WR-2721 tolerable to the patient and physician, i.e., an adequate therapeutic index.

The successful treatment of colorectal cancer requires agents with more activity than mitomycin, which produced no responses in this series of 97 patients. The merit of

WR-2721 will, therefore, have to be judged on the basis of its use in conjunction with other, more effective, chemotherapy regimens.

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