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Phase-I trial of combination therapy with continuous-infusion MMPR and continuous-infusion 5-FU

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Summary. *Fourty-four evaluable patients were treated with 6-methylmercaptopurine riboside (MMPR) at a dose of 20 mg/m²/day × 5 by continuous IV infusion (days 1–5) and 5-fluorouracil (5-FU) on an escalating dose schedule of 300–1519 mg/m²/day × 5 by continuous IV infusion (days 2–6). Dose-limiting oral mucositis occured at a 5-FU dose of 1,381 mg/m²/day; other toxicities included nausea, vomiting, diarrhea, skin rash, and occasional myelosuppression. A partial and a complete response were observed in two previously untreated patients with metastatic colon carcinoma given the highest 5-FU doses (1,381 and 1,519 mg/m²/day). Bone marrow phosphoribosyl pyrophosphate (PRPP) levels monitored after 24 h of MMPR treatment indicated increases of 7.8- and 9.2-fold those found prior to therapy.*

Introduction

5-Fluorouracil (5-FU) is an effective agent in the treatment of carcinomas of the breast and gastrointestinal tract [1, 5]. The incorporation of 5-FU into breast carcinoma RNA has been reported to be a major mechanism of action of this agent [8]. In vitro experiments modulating 5-FU metabolism have demonstrated that 5-FU incorporation into RNA can be significantly enhanced by several agents [3, 7,9]. *N*-Phosphonacetyl-L-asparate (PALA) reduces uracil nucleotide pools [11] and increases (5-FU) RNA formation [7, 9, 13]. This effect has been associated with increased cell lethality. The incorporation of 5-FU into RNA has also been enhanced by increasing intracellular levels of phosphoribosyl pyrophosphate (PRPP) and thereby phosphoribosyl transfer to 5-FU, with either methotrexate (MTX) or 6-methylmercaptopurine riboside (MMPR) [3, 7, 9].

Clinical trials with 5-FU have been directed at enhancing the activity of this agent by combination with PALA [2, 14, 15]. The administration of continuous-infusion PALA and bolus 5-FU has resulted in dose-limiting mucositis [14]. Myelosuppression was minimal with this regimen. Another phase I trial of continuous-infusion PALA and continuous-infusion 5-FU also resulted in dose-limiting mucositis [15]. Other toxicities included nausea, vomiting, skin rash, and occasional myelosuppression.

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Since intracellular PRPP levels are increased by exposure to MMPR, this agent thus provides another means of enhancing the formation of 5-fluorouracil ribonucleotides [7, 9]. Synergistic cytotoxicity of MMPR/5-FU combinations has been demonstrated in vitro [7, 9]. MMPR has also potentiated the antitumor activity of 5-FU in animal models [13].

The present study describes the results obtained in a phase-I trial of continuous-infusion MMPR and 5-FU.

Materials and methods

Clinical studies. Eligible patients had histologically confirmed malignancy refractory to standard therapy with a performance status of 2 or less on the ECOG scale, and had fully recovered from toxicity associated with prior treatment (at least 3 weeks since the prior therapy). Written, informed consent was obtained prior to therapy. MMPR (provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md) was administered throughout the study at a dose of 20 mg/m²/day × 5d by continuous infusion. The initial 5-FU dose was 300 mg/m²/day by continuous infusion for 5 days. This dose is based on one-fifth of the maximum tolerated dose of 5-FU (1,500 mg/m²/day × 5) [12]. Subsequently, the 5-FU dose was increased to 450, 560, 645, 710, 858, 944, 1,038, 1,144, 1,256, 1,381, and 1,519 mg/m²/day × 5. The 5-FU infusion began 24 h after initiation of the MMPR infusion at a separate venous site and continued for the succeeding 5 days. The treatment cycle was 28 days. If patients experienced moderately severe oral mucositis (unable to tolerate semi-solid food) for 7 days or longer the dose of 5-FU was reduced to the next-lower level on the dose escalation scale.

Bone marrow nucleated cell PRPP levels. Bone marrow samples were obtained from two patients prior to and after 24 h of MMPR therapy (before the initiation of 5-FU chemotherapy). Mononuclear cells were obtained by Ficoll-Hypaque separation and heated to 96° C for 45 s to inactivate cellular enzymes. The cellular content of PRPP was measured by [14C]-carbon dioxide release as described previously [6].

Results

Clinical study

In all, 51 patients were eligible for the study, but six were inevaluable for toxicity or response: three patients died early

Table 1. Patient characteristics

Number of patients (male/female)	51 (32/19)
Number of patients evaluable for toxicity	45
Total courses evaluable for toxicity	129
Ages of evaluable patients	
Range	37–76
Mean	57.1 ± 8.9
Median	57
Previous treatment	
Chemotherapy	24
Radiation	4
Chemotherapy and radiation	1
None	14

Table 2. Diseases among patients evaluable for toxicity

Colorectal	40
Ovarian carcinoma	4
Pancreatic carcinoma	1
Adenocarcinoma/gallbladder	1
Adenocarcinoma/renal	1
Prostate carcinoma	1
Adenocarcinoma/unknown primary	3
Total	51

Table 3. Toxicity

Dose 5-FU (mg/m^2/day)	No. of courses	0	1+	2+	3+	4+
Mucositis						
300	3	2	1			
450	4	4				
560	7	6	1			
645	12	11	0	1		
710	14	13	1			
858	12	9	2		1	
944	10	8	1	1		
1,038	18	17	1			
1,144	19	16	2	1		
1,256	12	9	2		1	
1,381	14	8	4		1	1
1,519	4	0	1	2	1	
Diarrhea						
300	3	3				
450	4	4				
560	7	7				
645	12	11		1		
710	14	12	1	1		
858	12	11	1			
944	10	9	1			
1,038	18	16	2			
1,144	19	18	1			
1,256	12	11	1			
1,381	14	12		1	1	
1,519	4	3		1		

of nontoxic causes and follow-up data were incomplete on the other three. The remaining 45 evaluable patients received 129 courses of therapy, with a mean of four courses per patient (median, 2 courses; range, 1–14 courses). The characteristics of the evaluable patients are presented in Table 1. In 78% of the patients the diagnosis was colorectal carcinoma, and four patients had ovarian carcinoma (Table 2). Prior 5-FU therapy had been administered to 14 of the patients, while 21 had received chemotherapy with other agents than 5-FU, including 5-fluorodeoxyuridine, mitoxanthrone, diethylstilbesterol, methyl-CCNU, *cis*-platinum, adriamycin, mitomycin C, and BCNU. Prior radiation therapy had been administered to 4 patients, predominantly to the pelvis, and nine patients had received both prior chemotherapy and radiation therapy. Only 14 patients had received no prior therapy.

Table 4. Tumor response

Dose	No. of courses	No. evaluable	Complete response	Partial response	Stable	Progression
300	3	2			1	1
450	4	2			2	2
560	7	5			1	4
645	12	7			4	3
710	14	9			5	4
858	12	8			7	1
944	10	6			3	3
1,038	18	16			8	8
1,144	19	16			12	4
1,256	12	9			5	4
1,381	14	7		1	4	2
1,519	4	2	1		1	

Toxicity was confined predominantly to the gastrointestinal tract (Table 3). Mucositis was the dose-limiting toxicity and occurred at a frequency of greater than 50% at or above a dose level of 5-FU of 1381 mg/m^2/day. Mucositis generally began on the 5th or 8th day of the treatment cycle and persisted for 5–14 days. Diarrhea was less severe and was associated with higher doses of 5-FU. An erythematous papular rash, predominantly over the face and upper trunk, occurred in one patient and resolved with discontinuation of therapy (5-FU: 1,256 mg/m^2). Central nervous system toxicity manifested in reversible confusion occurred in two patients. Myelosuppression (nadir of less than 1,000 cells/mm^3 on day 14) occurred in only six courses of treatment with 5-FU, all in patients with prior pelvic irradiation, and did not appear to be dose-related. Nausea and vomiting occurred in six courses. Superficial venous thrombophlebitis near the infusion site also occurred in four courses of treatment.

Eighty-nine courses of therapy were evaluable for response (Table 4). A complete response and a partial response was observed in two patients with metastatic colorectal carcinoma at a 5-FU dose of 1,381 mg/m^2 and 1,519 mg/m^2, respectively. Fifty-three courses were associated with stabilization of disease. In one case, disease stabilization persisted for over 1 year of therapy. Thirty-six courses of therapy were associated with the development of progressive disease.

The bone marrow nucleated cell PRPP levels were measured in two patients, before and 24 h after the initiation of treatment with MMPR. Bone marrow samples following 24 h of infusion with MMPR were found to contain 7.8 and 9.2 times the level of PRPP detectable prior to the initiation of therapy. These findings are consistent with data obtained with human breast carcinoma cells exposed to MMPR [9].

Discussion

The biochemical rationale for the combination of MMPR and 5-FU is based upon the concept that 5-FU incorporation in

tumor cell RNA can be enhanced by promoting the phosphorylation of 5-FU through increased intracellular levels of PRPP. Since RNA synthesis continues in cell cycle phases not devoted to DNA replication, the prolongation of cellular exposure to 5-FU by continuous infusion might be expected to result in enhancement of the drug effect. Previous studies with 5-FU given by continuous infusion have suggested the superiority of this mode of administration [12]. In this phase-I trial, continuous-infusion MMPR was given in combination with continuous-infusion 5-FU. Measurements of bone marrow PRPP levels suggest that the administration of MMPR to certain patients is capable of significantly increasing the intracellular levels of PRPP. In vitro experimentation has previously demonstrated that enhancement of 5-FU incorporation into RNA is promoted by elevated levels of PRPP [3, 7, 9].

In this study, dose-limiting toxicity was found with MMPR (20 mg/m^2/day × 5) and 5-FU above 1381 mg/m^2/day × 5. Diarrhea was also observed at higher doses. Other toxicities included skin rash, development of a confusional state, and occasional myelosuppression. The antitumor activity of this phase-I combination of 5-FU and MMPR was limited at lower 5-FU dose levels. However, a complete response and a partial response were observed with higher, but tolerable, doses of 5-FU (1,381 and 1,519 mg/m^2/day). Both responses occurred in previously untreated patients. The results of this study suggest that the toxicity with continuous infusion of both MMPR and 5-FU is similar to that encountered with similar doses of 5-FU alone. A direct comparison of MMPR/5-FU versus 5-FU alone in previously untreated patients with colorectal carcinoma will now be needed to determine whether MMPR enhances the activity of 5-FU against this disease. The results of the present clinical study and studies with PALA and 5-FU [4, 14, 15] suggest that combination chemotherapy with PALA/MMPR/5-FU might be clinically tolerated and should be evaluated in a phase-I trial.

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