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Oral dipyridamole and methotrexate in human solid tumors: a toxicity trial

Celestia S. Higano and Robert B. Livingston

Division of Oncology, Department of Medicine, University of Washington School of Medicine and Veterans Administration Medical Center, Seattle, Washington 98108, USA

Summary. Dipyridamole (DP) blocks nucleoside salvage by inhibiting uptake at the cell membrane. At the usual oral doses DP has no cytotoxic activity, but when combined with an antimetabolite, it results in synergistic cell kill in vitro. In this study, 45 patients with advanced solid tumors were treated with oral DP and i.v. or i.m. methotrexate (MTX) to define the toxicity of this combination. The DP dose was 75 mg b.i.d. in the first 16 patients, 150 mg b.i.d. in the next 2, and 75 mg q.i.d. in the remaining 27 patients. MTX was given weekly at an initial dose of 10–30 mg/m^2 and increased weekly by 5–10 mg/m^2 to the maximum tolerable dose (MTD) or a maximum of 60 mg/m^2; thereafter that dose was given every other week. DP levels ranged from 2.76 to 11.46 μM, with a mean of 5.67 μM in four patients taking 75 mg q.i.d. The combination of oral DP and MTX was generally well tolrated and did not appear to result in any more myelotoxicity or mucositis than that expected for MTX alone. One patient experienced severe headaches related to DP, ten patients experienced grade 3 or 4 neutropenia and/or thrombocytopenia, and four patients had grade 3 mucositis. Although this trial was not designed as a phase II study, one partial remission was observed in a patient with metastatic pleomorphic adenoma of the parotid gland and seven patients showed significant improvement.

Introduction

In his Clowes lecture, George Weber [8] called for the design of chemotherapy protocols based on the knowledge of the "biochemical strategy" of cancer cells. He presented data showing that inhibition of de novo biosynthesis of nucleotides by antimetabolites could be bypassed by the salvage of preformed nucleosides transported across the cell membrane and their subsequent conversion to nucleotides. Blockade of the so-called salvage pathway by dipyridamole (DP) at the level of the cell membrane, in combination with an antimetabolite, resulted in synergistic cell kill in vitro [8]. Based on these findings in vitro, we were interested in applying this approach in vivo.

Methotrexate (MTX), one of the oldest cytotoxic agents, blocks de novo nucleotide synthesis by interfering with folic acid metabolism. Its pharmacokinetics, toxicities, side effects, and response rates in a variety of tumor types have been well documented. Weekly administration of MTX has been extensively studied in patients with head and neck cancer; the toxicities associated with this dose schedule are well described [2].

DP is widely used in the clinical setting for its antiplatelet or coronary vasodilator effects. At levels that are achievable with oral dosing (<10 μM), DP alone has no cytotoxic activity in vitro [4]. As an inhibitor of facilitated transport systems such as those for nucleosides, it blocks the salvage pathway at the level of the cell membrane [9]. Unlike most other known inhibitors of the salvage pathway, this drug has been used in clinical practice for years and has minimal toxicity when given orally at therapeutic doses.

Modulation of the effects of MTX by DP might result in increased toxicity, particularly to platelets, as well as an increased antitumor effect. We initiated the following trial to determine the toxicity and possible clinical efficacy of this combination.

Patients and methods

From September 1983 through March 1986, 45 patients with advanced solid tumors were entered in the study. Eligibility criteria included a malignancy either refractory to conventional treatments or for which there was no established therapy. Patients were required to have measurable or evaluable disease and an Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 3. "Good-risk" patients had an estimated creatinine clearance of >60 ml/min, no evidence of third-space fluid collections, and no extensive prior therapy; "poor-risk" patients had impaired renal function (creatinine clearance of <60 but >30 ml/min), third-space fluid, and multiple prior chemotherapeutic regimens and/or pelvic or hemibody radiation therapy.

To achieve a steady-state concentration, oral DP was initiated in all patients at least 2 days prior to the initial dose of MTX. The first 16 patients received oral DP at 75 mg b.i.d. Previous studies indicated that this dose would result in a mean peak concentration at steady state of 1.7 μg/ml (or 3.3 μM) [3]. As in vitro studies described DP-antimetabolite synergy at concentrations between 1 and 10 μM [1, 5], the dose of DP was increased when no untoward toxicity was noted at the 75-mg b.i.d. level. The next two patients received 150 mg b.i.d. Since this

Offprint requests to: C. S. Higano, VA Medical Center, 1660 S. Columbian Way, Seattle, WA 98108, USA

Table 1. Patient characteristics

Median age years (range):	55 (19–79)
	Number of patients
Total	45
Sex	
male	24
female	21
Good risk	30
Poor risk	15
Previous therapy	
chemotherapy	24
radiation	3
chemotherapy + radiation	10
none	8

Table 2. Treatment and toxicity data

	Overall	(Good risk/ Poor risk)
Treatment		
Median number of cycles/patient	6	(8/3)
Median dose MTX mg/m^2	50	(50/20)
Toxicity[2]		
Neutropenia		
grade 3	5	(1/4)
grade 4	5	(1/4)
Thrombocytopenia		
grade 1	2	(0/2)
grade 2	2	(1/1)
grade 3	6	(1/5)
grade 4	4	(0/4)
Mucositis		
grade 1	5	(4/1)
grade 2	4	(3/1)
grade 3	4	(1/3)
DP-related symptoms		
headache	4	(2/2)
orthostatic blood pressure	2	(1/1)
nausea	7	(5/2)
malaise, fatigue	3	(2/1)

[2] ECOG criteria

dose was not well tolerated (one patient experienced severe headache, the other, orthostatic hypotension and nausea), the remaining 27 patients received 75 mg q.i.d. for 12 doses per cycle, starting 2 days prior to chemotherapy administration and ending approximately 12 h after the MTX dose.

MTX was given parenterally (i.v. or i.m.) on an outpatient basis. Good-risk patients received an initial dose of 30 mg/m^2, with a weekly increment of 10 mg/m^2 to toxicity (myelosuppression and/or mucositis) or a maximal dose of 60 mg/m^2. Thereafter the MTX was given every other week. Poor-risk patients started at a lower initial dose of 10–15 mg/m^2, with a weekly increment of 5–10 mg/m^2 as tolerated to toxicity or a maximal dose of 60 mg/m^2, the maximum tolerated dose being given every other week.

A complete blood count with differential and platelet count and a serum creatinine determination were obtained prior to each dose of MTX. Provided that renal function was unchanged, that the absolute neutrophil count was $>1500/mm^3$ and the platelet count was $>100{,}000/mm^3$, and that there was no evidence of mucositis, the scheduled dose of MTX was given. The dose was reduced one level (10 mg/m^2) according to nadir counts (absolute neutrophil count of $<1000/mm^3$ and/or platelet count of $<75{,}000/mm^3$) and/or the development of moderate mucositis.

Patients were assessed for response no less frequently than every 4 weeks. Response was defined as stable (a steady state or response less than partial remission or progression for a minimum of 4 weeks), improved (tumor shrinkage of $<50\%$ for a minimum of 4 weeks), partial remission (a $\geqslant 50\%$ decrease in the sum of the product of the greatest perpendicular diameters of all objectively measurable lesions; significant improvements in all evaluable tumor sites), or progressive disease (an unequivocal increase of 25% in the size of any measurable lesion, the appearance of new lesion(s), or definite increase in evaluable disease).

DP levels were obtained in four patients. After 2 days on DP alone, a peak level was drawn on the 3rd day 1.5–2 h after the oral dose taken prior to MTX administration. DP levels were determined by HPLC by a technique modified from that described by Wolfram and Bjornsson [11].

Results

The clinical characteristics of the 45 patients entered in the study are summarized in Table 1. In all, 34 patients had received prior chemotherapy with or without radiation, 3 had undergone radiation therapy, and 8 had received no previous treatment. The treatment and toxicity data on all patients is presented in Table 2. Hematologic toxicity generally occurred in patients who were heavily pretreated and/or when MTX was given in the face of dehydration suggested by an elevation in serum creatinine and blood urea nitrogen (BUN) levels. Only one patient required admission for fever and neutropenia; this patient had *E. coli* sepsis and had developed progression of a right middle-lobe syndrome found at the time of diagnosis. All but one patient who developed a thrombocytopenia of $<50{,}000/mm^3$ had been heavily pretreated (more than two prior chemotherapy regimens and/or pelvic or hemibody irradiation). There were no episodes of life-threatening bleeding, although three patients received prophylactic platelet transfusions; one of these was given MTX despite a platelet count of $<75{,}000/mm^3$.

In all 35 patients who received four or more doses of MTX (1 month or more of treatment) were considered evaluable for response. Table 3 shows the tumor types for all patients and their respective responses. Despite progressive disease in 19 of 35 patients evaluable for response, 15 patients had either stable or improved status. There was one partial remission in a previously treated patient with disease measurable by chest X-ray.

DP levels were obtained in four patients; one had a single peak level and three had serial peak levels obtained with successive cycles of therapy. Figure 1 shows the distribution of levels for each patient. All patients were on the 75-mg q.i.d. schedule of DP. The levels ranged from 2.76 to 11.46 μM, with a mean of 5.67 μM (the patient with the highest levels reported no side effects).

Table 3. Tumor types and response data

Tumor type	*n*	NE	PD	STA (months)	IMP (months)	PR (months)
Carcinoma						
colon	11	1	7	2 (3, 4)	1 (7)	
lung, non-small-cell	6	4		2 (3, 4)		
breast	5	1	3	1 (2)		
pancreas	3			1 (3)	2 (4, 7)	
head and neck	2		2			
unknown	2	1	1			
Mesothelioma	5	1	1		3 (3, 7, 8)	
Sarcoma						
osteogenic	3	1	2			
leiomyosarcoma	2			1 (6)	1 (30+)	
chest wall	1		1			
alveolar	1		1			
Other						
pseudomyxoma	1				1 (7)	
melanoma	2	1	1			
parotid[a]	1					1 (11)
Total	45	10	19	7	8	1

NE, not evaluable, i.e., received fewer than four cycles of treatment; PD, progressive disease; STA, stable disease; IMP, improved; PR, partial remission; [a] pleomorphic adenoma

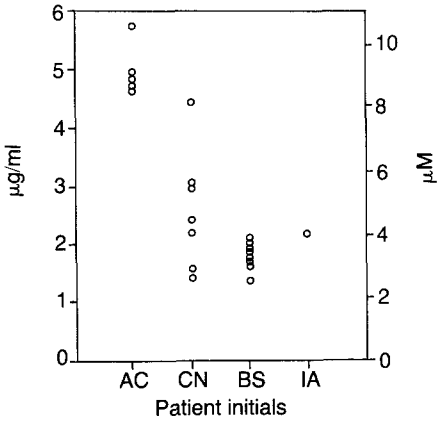

Fig. 1. Peak total DP measurements were made in four patients 1.5–2 h after the tenth dose of oral DP. Each point represents the DP level prior to one cycle of MTX

Discussion

Subsequent to the initiation of this trial, several clinical studies were carried out to examine the modulation of MTX by DP. Subar et al. [7] reported that oral DP at 50–70 mg q.i.d. enhances the mucosal and hematologic toxicity of low-dose oral MTX (2.5 mg b.i.d. for 2–5 days, given every 3 weeks). Unfortunately, the toxicity of this schedule of MTX alone is unclear, as this is not a commonly used regimen; therefore, it is difficult to conclude that DP actually modulated MTX. Wilson et al. [10] studied the effect of continuous-infusion i.v. DP on MTX toxicity and pharmacology. Using patients as their own controls, they demonstrated very clearly that DP infusion modulates MTX cytotoxicity presenting as mucositis and/or myelosuppression and that this effect was not due to an alteration in MTX pharmacokinetics [10]. A third study [12] examined i.v. MTX and oral DP at a dose of 50 mg q.i.d. The mean total DP level was 3.1 μ*M*, with a range of 0.2–9.8 μ*M*, in 12 patients. As in the continuous-infusion study this dose of oral DP did not alter MTX kinetics. No added toxicity was noted at a dose of 40 mg/m^2 MTX [12].

In the present study, the combination of MTX and oral DP was generally well tolerated. Mucositis and myelosuppression did not appear to be greater than that expected for MTX alone at the same doses when compared with toxicity data in head-and-neck cancer patients receiving MTX weekly. The toxicity of oral DP most frequently consisted of transient headache and/or mild nausea, sometimes accompanied by orthostatic hypotension. Two consecutive patients were treated with 150 mg DP b.i.d.; both tolerated this dose poorly, and one was removed from the study due to severe headache even at a dose of 75 mg q.i.d. Thereafter, all patients were put on the 75-mg q.i.d. schedule without further attempts at dose escalation.

Total DP levels measured in four patients ranged from 2.76 to 11.46 μ*M*, with a mean of 5.67 μ*M*. Such values are within the synergistic range reported in experimental systems. However, the levels reported from in vitro studies probably reflect a greater proportion of free DP, since this drug is extensively protein-bound in vivo. The significance of DP plasma protein binding in vivo remains uncertain, but it is possible that the levels of free drug in our study were inadequate to test the modulation hypothesis.

As might be expected, the toxicity of this regimen is enhanced in the presence of dehydration and in poor-risk patients, particularly those who had previously received extensive pelvic or sequential hemibody irradiation.

Although it is not possible to draw conclusions regarding response data in a phase I study of a heterogeneous population of previously treated patients, some interesting observations can be made. First, of three patients treated for pancreatic cancer, two exhibited improvement for 4 and 7 months; both had liver metastases at the time of diagnosis. One patient with a performance status of 3 due to

pain became fully ambulatory, and a computerized axial tomography (CAT) scan showed tumor shrinkage, although not enough to qualify this improvement as a partial remission. Response of any kind in a tumor that is generally considered to be refractory is intriguing in light of the in vitro data reported by Salmon [6], in which marked potentiation of MTX by a DP analog, RA-233, occurred in three pancreatic cancer lines. In the same study, sarcoma colony formation was also sensitive to this combination. In the present study, three of four evaluable patients with mesothelioma showed objective improvement, evident by chest X-ray and/or CAT scan, for 3, 7, and 8 months. Of two patients with leiomyosarcoma, CAT scans showed evidence of tumor regression in one, who remains improved at 30+ months on study. The other patient had a 6-month stabilization of disease, which was progressing on adriamycin at the time of entry in the study. Finally, a previously treated patient with pleiomorphic adenoma of the parotid gland with biopsy-proven lung metastases had a partial remission that lasted for 11 months.

In this trial, oral DP at 75 mg q.i.d. was combined with i.v. or i.m. MTX at standard doses without unexpected toxicity. There were no life-threatening bleeding or infectious episodes related to myelosuppression. The observed evidence of an antitumor effect in pancreatic cancer, mesothelioma, leiomyosarcoma, and a salivary gland tumor is provocative and should be considered in planning phase II studies with MTX or other antimetabolites in combination with DP. Future studies exploring the modulatory effects of DP should include detailed pharmacologic evaluation to gather more information on the kinetics of free DP and determine whether there is a dose-response relationship. The question as to whether the therapeutic index of MTX or other antimetabolites can be improved by the addition of DP may be answered by phase III studies.

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