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Sequential inhibition of polyamine synthesis

A phase I trial of DFMO (α-difluoromethylornithine) and methyl-GAG [methylglyoxal-bis(guanylhydrazone)]

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Summary. *Both DFMO and methyl-GAG inhibit sequential enzymatic reactions in the pathway of polyamine biosynthesis. Since polyamines may be important factors in proliferation of cancer cells, we initiated a phase-I study of these agents in patients with advanced cancer. DFMO was given by mouth at a constant daily dose of 4 g/m^2 starting on day 1 of the treatment protocol. The dose of methyl-GAG ranged from 200 to 700 mg/m^2 administered IV every 2 weeks beginning on day 4. Twenty-two patients were entered into the protocol. Toxic reactions to this therapy were dose-related and included nausea, fatigue, diarrhea, and myelosuppression. One patient with colon cancer experienced a $>50\%$ decrease in measurable disease but developed severe myelotoxicity. While DFMO was well tolerated, the combination of drugs appeared to cause substantially more hematologic and gastrointestinal toxicity than encountered during our recent experience with methyl-GAG used alone. We suggest that future studies of this drug combination carefully evaluate levels of polyamines and inhibition of enzymatic activity to minimize toxicity.*

Introduction

Increased production of the natural polyamines (putrescine, spermidine, and spermine) has been correlated with early relapse and disease activity in patients with a variety of neoplastic disorders [4, 7]. Moreover, depletion of polyamines by drugs which inhibit their synthesis causes inhibition of growth and proliferation of tumor cells in vitro [10, 11]. The anticancer drug methyl-GAG (MGBG) produces a wide variety of biologic effects, but the exact mechanism whereby it exerts its cytotoxic effect is uncertain. Although MGBG inhibits an important enzyme in the pathway of polyamine biosynthesis (*S*-adenosylmethionine decarboxylase), the inhibition is reversible and recovery of polyamine synthesis occurs rapidly [6]. DFMO is a new agent which irreversibly inhibits the preceding, rate-limiting reaction in polyamine synthesis – namely the conversion of ornithine to putrescine by ornithine decarboxylase [11]. Experiments in vitro [5] and in vivo [2] have shown that the combination of MGBG with DFMO achieves a substantially greater cytotoxic effect than either agent used alone.

Following the favorable report by Janne et al. [8] who used MGBG and DFMO in a small series of patients with acute lymphoblastic leukemia, we initiated a phase I evaluation of this drug combination in patients with advanced cancer.

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Patients and methods

Patients with advanced cancer refractory to conventional treatment were eligible for entry into this trial. All patients had histologically confirmed disease, performance status ≥ 50 (Karnofsky), adequate renal and hepatic function (defined as serum bilirubin and creatinine concentration ≤ 1.5 mg/dl), and adequate hematologic reserve (defined as total leukocyte (WBC) count $\geq 4{,}000$ cells/mm^3 and platelet count $\geq 140{,}000$ cells/mm^3). Patients had not received other chemotherapy or radiation within 3 weeks before entry and all had recovered from toxic effects of previous treatment. Absence of bidimensionally measurable parameters of disease or prior therapy with MGBG did not exlude entry into this phase-I trial.

Pretreatment evaluation included a history and physical examination, complete blood count, 12-channel serum biochemical profile, chest roentgenogram, and audiometric testing. Additional radiographic or radionuclide procedures were performed as needed to assess the extent of disease. All patients gave signed informed consent and this study was approved in advance by this Center's Institutional Review Board. Conventional response and toxicity criteria were observed [14].

The study was designed to evaluate the effects of increasing doses of MGBG using a fixed dose of DFMO. DFMO was administered on a continuous daily basis starting on day 1 of the treatment protocol. The total daily dose of 4 g/m^2 was divided into four equal amounts, each diluted with 50 cm^3 water and taken by mouth every 6 h. MGBG was administered on day 4 and every 2 weeks thereafter. We did not allow dose escalation of MGBG within the same individual. The prescribed dose of MGBG was diluted in 250 ml 5% dextrose solution and infused IV over 1 h. Patients who developed unacceptable toxicity or who manifested progressive disease were removed from the study.

Results

Twenty-two patients with advanced cancer were entered onto this protocol. The diagnoses and pretreatment characteristics of this patient population are presented in Table 1. With the exception of two patients with prostatic cancer who had received only hormonal treatment, all patients had received prior chemotherapy with or without additional radiation. Table 2 indicates the number of patients entered at each dose level of MGBG and the number of doses of MGBG each patient received. Two patients entered at the lowest dose level

Table 1. Characteristics of patients treated with DFMO/MGBG

Median age, years (range)	56 (22–78)
Median performance status, Karnofsky (range)	60 (50–80)
Median no. of prior chemotherapy courses	3 (0–10)
Median no. of prior chemotherapy drugs	6 (0–16)
Histologic diagnoses (n = 22):	No. of patients
Malignant lymphoma	12
Prostate	5
Colorectal	2
Myeloma	1
Carcinoid	1
Bladder	1

Table 2. Phase-I study: number of MGBG doses administered

MGBG dose level (mg/m^2)	No. of patients entered	No. of MGBG doses			
		0	1	2	3 (+)
		No. of patients			
200	8	2	1	4	1
400	4	–	–	3	1
600	6	–	–	4	2
700	4	–	2	2	–

Table 3. Hematologic toxicity of DFMO/MGBG

MGBG dose level (mg/m^2)	No. of patients entered	No. of patients evaluable	Median WBC nadir[a] (range)	Median platelet nadir[a] (range)	Median change Hgb conc.[b] (range)
200	8	6	5.1 (3.7–11.9)	214 (133–389)	−1.7 (−0.1 to −2.4)
400	4	4	5.0 (4.4–10.9)	353 (133–572)	−2.3 (−0.3 to −3.8)
600	6	6	5.4 (0.2–19.5)	117 (14–547)	−1.8 (−0.9 to −4.8)
700	4	4	2.3 (1.0– 8.5)	84 (18–436)	−0.6 (−0.3 to −1.5)

[a] Data expressed as 1,000 cells/mm^3
[b] Data expressed in g/dl

Table 4. Number of patients who experienced non-hematologic toxicity from DFMO/MGBG

Reaction	Dose of MGBG[a]			
	200 (6)[b]	400 (4)	600 (6)	700 (4)
Fatigue (≥ 2+)	1	2	3	3
Vomiting	–	1	2	3
Diarrhea	1	1	1	1
Myalgia	–	2	–	–
Mucositis	–	–	–	1
Skin eruption	1	–	–	–

[a] Drug dose expressed as mg/m^2
[b] Numbers in parentheses indicate number of patients evaluable at each dose level

developed acute medical complications related to their underlying disease and were removed from the study within the first 4 days prior to receiving the initial dose of MGBG.

Toxicity

Oral DFMO at the constant daily dose of 4 g/m^2 was well tolerated. The only complaints voiced by patients concerning this medication related to its salty taste and the frequency with which the drug was administered (every 6 h). No patient complained of decreased auditory acuity, an effect which has been reported following high doses of DFMO [12].

Toxic effects of this combination generally increased in frequency and severity with increasing doses of MGBG. Myelosuppression was dose-limiting with this drug combination. Hematologic data are presented in Table 3. While there was quite a broad range of tolerance to the myelosuppressive effects of these drugs, there appeared to be a dose-related decrease in WBC and platelet counts. Two patients with severe thrombocytopenia (< 20,000/mm^3) (one each at the 600 and 700 mg/m^2 dose level) developed gastrointestinal hemorrhage which necessitated hospitalization and transfusion. Two patients with prostatic cancer also developed hematuria although they were not significantly thrombocytopenic. Two patients (one each at 600 and 700 mg/m^2) required hospitalization for fever related to infections. Anemia was observed frequently during this trial, although its relation to drug toxicity is uncertain given the advanced nature of disease in these patients.

Non-hematologic toxicity is described in Table 4. Fatigue, nausea, and diarrhea occurred commonly and the severity of these effects was also dose-related. Protracted vomiting persisting for longer than 24 h occurred in three of the four individuals treated at the 700 mg/m^2 level of MGBG. Two of these patients refused further treatment because of the severity of this reaction. The fatigue/weakness syndrome which commonly occurs following more intensive treatment with MGBG [13] was frequently noted in this trial, generally after the first dose. One patient with mycosis fungoides who received a single dose of MGBG at 200 mg/m^2 developed a severe erythematous reaction of her entire skin surface, resembling a 'scalded skin' syndrome. This patient had received electron beam therapy ot her skin; however, this reaction had not occurred with the prior use of MGBG as a single agent nor was it associated with any therapeutic effect.

Therapeutic results

One patient with advanced colon cancer and extensive hepatic metastases achieved a > 50% decrease in the extent of his measurable disease, accompanied by a decrease in carcinoembryonic antigen levels and improvement in serum levels of hepatic enzyme activity. This improvement occurred after the first injection of MGBG at a dose of 700 mg/m^2. However, the second injection produced severe myelotoxicity and debilita-

tion. The patient declined further therapy and died suddenly 8 days later. Despite the therapeutic activity of MGBG in patients with advanced malignant lymphoma [9, 14], none of the 12 patients with various types of lymphoma who were treated with this protocol responded.

Discussion

The recent re-evaluation of MGBG has shown that this drug can be safely administered using intermittent dose schedules and that it has definite anticancer activity, particularly in patients with relapsed malignant lymphoma [9, 14]. However, it is clear that intensive treatment with this agent causes unacceptable toxicity. Because of its unique mechanism of action, a variety of attempts have been made to extend the activity of MGBG.

MGBG appears to compete with spermidine for uptake into tumor cells [3]. DFMO irreversibly inhibits ornithine decarboxylase and thus causes depletion of spermidine [11]. Exposure of tumor cells to DFMO increases intracellular uptake of MGBG [1]. Moreover, we have shown that the DFMO/MGBG combination can produce synergistic cytotoxicity in vivo without increased toxicity [2, 4]. Janne et al. [8] were the first to use these drugs in combination and reported favorable results in five children with refractory acute lymphoblastic leukemia. Our study sought to explore the biologic effects of these drugs using varying doses of MGBG combined with a fixed dose of DFMO that reduced excessive polyamine excretion in a patient with melanoma [12].

With the exception of the severe skin reaction sustained by a patient with mycosis fungoides, we observed no toxic reactions in this study which have not been recorded for MGBG used alone [14]. However, based on extensive single-agent experience with MGBG [13, 14], DFMO appeared to markedly potentiate this toxicity, particularly myelosuppression – an effect not commonly seen when MGBG is administered every 2 weeks [13]. Moreover, continuation of DFMO through the period of myelosuppression appeared to delay recovery of hematopoiesis in several patients relative to those individuals in whom the drug was stopped because of myelotoxicity.

In view of the substantial toxicity observed in this trial, we do not recommend further study with DFMO and MGBG using this fixed dose and schedule. However, the concept of polyamine depletion in cancer chemotherapy remains attractive in view of its potential selectivity against rapidly dividing neoplastic cells. Conceivably, short 'priming' courses of DFMO followed by single injections of MGBG repeated at appropriate intervals might more fully exploit this mechanism and allow recovery of hematopoiesis. Since measurements of polyamines and ornithine decarboxylase activity are now available in many research laboratories, current investigation should define that dose of DFMO which maximally inhibits ornithine decarboxylase, thus maximally depleting spermidine. We recommend that further studies of this drug combination employ routine biochemical monitoring of these parameters to avoid the toxicity observed in this trial.

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