Eur J Cancer Clin Oncol, Vol. 23, No. 6, pp. 683–687, 1987.
Printed in Great Britain

0277–5379/87$3.00 + 0.00

Reversal of Cancer Chemotherapeutic Resistance by Amphotericin B—A Broad Phase I–II Pilot Study

CARY A. PRESANT, PHYLLIS MULTHAUF and GERALD METTER

Los Angeles Oncologic Institute, Wilshire Oncology Medical Group, Los Angeles, California (CAP), and City of Hope National Medical Center, Duarte, California, U.S.A.

Abstract—*In order to determine if it was possible to reverse clinically evident chemotherapeutic drug-resistance, 51 evaluable patients received chemotherapy (in doses and schedules on which they had previously demonstrated tumor progression) together with amphotericin B (AMB). AMB was given in 1-, 2-, or 4-day courses. There was 1 complete response (2%), and 5 partial responses (10%). Response rates tended to be higher in the 4-day treatment program (23%) than in the 1- or 2-day AMB treatment schedules (8%). Toxicity was that expected with chemotherapy (myelosuppression), or AMB alone (fever, chills, and reversible mild azotemia). We conclude that AMB is only infrequently able to reverse clinical drug-resistance, but that this might have palliative effects in a small number of patients in whom other standard chemotherapeutic drugs lack clinical effectiveness.*

INTRODUCTION

In 1977, we reported our initial results in 7 patients who had received combination chemotherapy together with amphotericin B (AMB) which suggested that AMB could reverse clinical resistance to combination chemotherapy [1]. Since that time, we have reported the results with successive patients who received AMB in an attempt to reverse established clinical resistance to chemotherapeutic agents [2, 3]. We now report our final evaluation of 55 patients who have been studied during a trial of AMB plus chemotherapy.

METHODS

Patients were eligible for this treatment program if they had biopsy-proven malignant disease and had given voluntary informed consent prior to participation in the study. They were required to have measurable metastatic tumor, and to have shown progression of tumor size despite at least 2 courses of the therapy to be combined with AMB.

Furthermore, previous trials of the chemotherapeutic agent to which resistance had been observed was required to have been associated with some evidence of drug toxicity, indicating that reasonable doses of the chemotherapeutic agent had been administered. Patients were required to have normal renal and hepatic function as well as a pretreatment leukocyte count of greater than 3500/mm^3, and a pretreatment platelet count of greater than 75,000/mm^3. The patients reported at this time include all patients previously reported [1–3].

THERAPEUTIC REGIMEN

All patients in this study received 1, 2, or 4 days of AMB, and following the completion of the final administration of AMB, received chemotherapy. Initial trials used 4 days of AMB, and latter studies explored 1- or 2-day schedules. The chemotherapy consisted of the same drugs to which patients had previosly demonstrated clinical resistance, in the same doses and schedules. No patient received chemotherapy in the same dose at which they had previously demonstrated severe or life-threatening toxicity.

The types of chemotherapy administered are detailed in the Results section. AMB in a 1-day course was administered at a dose of 40 mg/m^2 intravenously in 5% dextrose and water over a 4-hr to 6-hr period. In the 2-day dosage regimen, patients received 7.5 mg/m^2 AMB on day 1 and 30 mg/m^2 on day 2. In the 4-day treatment program, patients received 7.5 mg/m^2 AMB on day 1, 15 mg/

Accepted 21 October 1986.
Address reprint requests to: Dr. Cary A. Presant, Los Angeles Oncologic Institute, Wilshire Oncology Medical Group, 201 South Alvarado Street, Los Angeles, California 90057, U.S.A.

m^2 on day 2, and 30 mg/m^2 on days 3 and 4. Patients were premedicated with diphenhydramine and acetaminophen in order to control chills and fever; 100 mg hydrocortisone was added to the infusion to eliminate phlebitis. If chills were observed despite this therapy, meperidine was administered intravenously at a dose of 25 mg.

If patients had severe toxicity from the chemotherapeutic agent, the doses in the subsequent course were reduced by 50%. Patients who had severe hypokalemia or renal dysfunction or unusual cardiac responses (hypotension or tachycardia) were considered to be non-responders, and received no further AMB. If hypotension or tachycardia was observed, the AMB infusion was discontinued.

Chemotherapy was repeated at the same schedule as had been previously used for the chemotherapeutic agent. In most cases the therapy was repeated every 3–4 weeks.

EVALUATION OF THE ANTITUMOR RESPONSE

A complete response was defined as disappearance of all manifestations of tumor for longer than 4 weeks; partial response, a decrease in the sum of the products of the perpendicular diameters of all measured tumors by 50% for greater than 4 weeks, without the appearance of new tumors; stable, response less than partial response, but no progression; progression, appearance of new manifestations of tumor, a greater than 25% increase in the measured tumor area, or death of the patient during therapy.

RESULTS

Of the 55 patients entered into the trial, 51 were evaluable for antitumor response. The remaining 4 patients had incomplete documentation of tumor size, inadequate data collection, or had received additional agents to which resistance had not previously been demonstrated.

Fifty-one patients were evaluable for antitumor response (Table 1). Most patients were female between the ages of 40 and 70 years of age. The pretreatment Karnofsky performance status was 80% or greater in half of the patients. In the others, it was usually 60 or 70%.

Of the 51 patients evaluable, there was 1 complete response, 5 partial responses, and 7 patients with stable disease. The response rates were 2% complete response, and 10% partial response, for an overall response rate of 12%.

Only 1 patient had received AMB on a 1-day treatment schedule, and this was a patient with acute myelocytic leukemia who was previously reported [1]. Thirty-seven patients received AMB in a 2-day schedule; there were 2 partial responses (5%).

The overall response rate for 1- or 2-day AMB was 8%. Thirteen patients received the 4-day treatment program with AMB and 3 had partial responses (23%).

There were 17 different types of tumors treated in this broad phase II study (Table 2). Of 10 patients with carcinoma of the breast, 3 had partial responses. There was 1 partial response each in soft tissue sarcoma, and multiple myeloma, 1 complete response in acute myelomonocytic leukemia. Twenty-three patients received AMB in combination with single agents (Table 3). Only 1 partial response was obtained, that being observed with AMB in combination with vinblastine.

All the remaining patients received combination chemotherapy, usually consisting of doxorubicin-containing combination regimens. Three partial responses and 1 complete response were observed in patients treated with the combination doxorubicin, BCNU, and cyclophosphamide, the combination which was originally reported [1].

A partial response was also observed in a patient with soft tissue sarcoma treated with actinomycin D, cyclophosphamide, and vincristine.

The duration of the complete response in a patient with acute myelomonocytic leukemia was 3 months. The durations of partial response were 1 month, 1 month, 2 months, 5 months, and 25 months.

Toxicity was predictably moderate, since we had previously observed that AMB did not increase chemotherapeutic toxicity of our initial chemotherapeutic regimen tested [1] and since patients were not treated with chemotherapy at doses which had produced toxicity previously. Nine per cent of the patients had a decrease in hemoglobin concentration greater than 3 g/dl, although all responded to transfusions and did not have progressive hemolytic disease. One-third of the patients had a granulocyte nadir less than 150,000/mm^3 which lasted less than 1 week.

Only 20% of the patients had thrombocytopenia, with a platelet count nadir less than 100,000/mm^3, and this was usually associated with patients who were receiving a nitrosourea. Stomatitis was observed in only 1 patient, and vomiting lasting more than 2 days was observed in only 1 patient.

The toxicity of AMB was predictable (Table 4); reversible azotemia with a blood urea nitrogen greater than 25 mg/dl was oberved in 9% of patients. One patient developed hypotension and bronchospasm, and 1 developed hypokalemia. The patients who developed hypotension also had tachycardia, suggesting that this was secondary to vasodilatation associated with fever. With the addition of hydrocortisone to the infusion, no patient had severe phlebitis. It is to be noted that prednisone or hydrocortisone were not continued in any of these patients other than the addition of hydrocortisone to the infusion bottle for the 4–6 hr period.

Table 1. *Patient characteristics and response*

Characteristic	No. patients	Response CR	PR	Stable	Progression or death
Total	51	1	5	7	38
Sex					
Male	16		2	3	11
Female	35	1	3	4	27
Age					
20–40	9	1	1	2	5
40–60	23		2	3	18
> 60	18		1	2	15
Pretreatment performance status					
80–100	25	1	2	6	16
< 80	21		2	1	18
Not recorded	5		1	1	3
AMB schedule					
1-day	1	1			
2-day	37		2	6	29
4-day	13		3	1	9

Table 2. *Tumor type and response*

Tumor type	No. patients evaluable	Response CR	PR	Stable	Progression or death
Breast	10		3		7
Colon	7				7
Lung					
Non-small cell	6			1	5
Small cell	1				1
Soft tissue sarcoma	7		1		6
Leukemia					
Acute myelomonocytic	1	1			
Chronic myelocytic, blastic	1			1	
Multiple myeloma	4		1	1	2
Kidney	3				3
Head–neck	3			1	2
Thyroid	2			1	1
Ovary	2				2
Stomach	1				1
Endometrium	1				1
Testes (mixed)	1			1	
Carcinoma of unknown primary	1			1	
Total	51	1	5	7	38

DISCUSSION

A critical problem facing the clinical oncologist and those involved in developmental therapeutics is overcoming drug resistance. The initial preclinical investigations of AMB were of particular clinical interest. These studies indicated that AMB, which combines with cholesterol in cellular membranes, was able to increase cellular uptake of a number of different molecules, including antifungal agents [4], antitumor agents [5], and even larger molecules such as fragments of DNA [6]. This was found in experimental animals to result in potentiation of antitumor activity *in vivo* [7, 8] and was most marked with certain nitrosoureas, doxorubicin, and alkylating agents.

This study indicates that the frequency with which AMB can reverse established resistance to other chemotherapeutic agents is low. Indeed, only 12% of patients had antitumor responses. Since most of the patients had been resistant not only to the chemotherapeutic agents employed, but also to 3 or 4 other types of chemotherapy, this highly

Table 3. Therapy and response

	No. patients evaluable	Response CR	PR	Stable	Progression or death
Single agent					
Doxorubicin	9			2	7
CCNU	6				6
Chlorozotocin	2				2
5-Fluorouracil	2				2
Vinblastine	2		1		1
Cis-platinum	1			1	
Actinomycin D	1				1
Combinations*					
ABC	10	1	3	1	5
ABCP	2			1	1
ABCBlVi	1			1	
AC	4				4
AD	1				1
AMCCc	1				1
ACc	2				2
ACD	1				1
ACM	1				1
ACBlVe	1				1
AFMi	1				1
AcCVi	1	1			
MF	1				1
VeViP	1			1	

*A, Doxorubicin; B, BCNU; C, cyclophosphamide; Bl, bleomycin; P, prednisone; Cc, CCNU; M, methotrexate; Ve, vinblastine; Vi, vincristine; F, 5-fluorouracil; Mi, mitomycin C; D, dacarbazine; Ac, actinomycin D.

Table 4. Toxicity

Toxicity	Incidence (%)
Hemoglobin fall > 3 g/dl	9 (4/46)
Granulocyte nadir < 1500 mm^3	33 (15/46)
Platelet nadir < 100,00/mm^3	20 (9/46)
Moderate or severe chills and fever	24 (11/46)
Reversible azotemia (BUN > 25 mg/dl)	9 (4/46)
Hypotension	4 (2/46)
Severe stomatitis	2 (1/46)
Bronchospasm	2 (1/46)
Hypokalemia	2 (1/46)
Prolonged vomiting > 2 days	2 (1/46)

resistant patient population was not an ideal setting in which to test the activity of AMB.

Nevertheless, it is evident that AMB does not have the degree of clinical activity suggested in the experimental mouse models [7, 8]. In those models, tumor cell killing by CCNU and doxorubicin was potentiated more than 500-fold by AMB. We estimate that clinical potentiation in those 18% with any measurable response was probably no more than 5–10-fold.

These results are consistent with those at MD Anderson Hospital [9] in which a low frequency of antitumor response was observed in patients heavily resistant to combination chemotherapy with 5-FU, doxorubicin and cyclophosphamide. A small amount of activity was nevertheless observed.

We have also tested AMB in other clinical settings. Despite our initial suggestive evidence that AMB was able to enhance antitumor responses in carcinoma of the lung [10, 11], a follow-up randomized study of chemotherapy utilizing doxorubicin, CCNU, hexamethylmelamine, and methotrexate with or without AMB has indicated that in patients with non-small cell carcinoma of the lung, AMB increased the antitumor response rate but decreased survival [13].

A randomized Southeastern Cancer Study Group evaluation of doxorubicin, cyclophosphamide, and methotrexate with or without AMB in patients with metastatic soft tissue sarcomas indicated no increase in response rate and no increase in survival [14]. And lastly, a phase II trial of AMB with CCNU (the drug most dramatically potentiated by AMB in experimental models—Reference [8]) showed no potentiation of antitumor responses in patients with renal or colorectal carcinomas [15]. These studies were all performed in tumors poorly responsive to chemotherapy.

We therefore conclude that AMB is occasionally able to reverse clinical drug resistance. AMB may have a limited role in providing additional palliation for selected patients with advanced disease. Future experimental trials to reverse drug resistance might focus on other polyenes, or use of the 4-day AMB regimen together with doxorubicin, BCNU and cyclophosphamide in more drug-responsive tumors (lymphoma, breast cancer, or small cell carcinoma of the lung).

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