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Phase I Study of 5-Azacytidine (NSC-102816)^{1,2,3,4}

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J. F. Laclius,⁵ and R. E. Bellet⁶

SUMMARY

Thirty patients with various solid tumors were treated with 5-azacytidine. Total doses ranged from 1.0 to 24.0 mg/kg and were given over a minimal period of 8 days. The major toxic effect was hematologic with significant leukopenia and thrombocytopenia usually occurring 20–30 days after the start of therapy, especially at higher dose levels. The marrow depression lasted 1–5 weeks and was fully reversible. Nausea and mild diarrhea were common following injection of the drug. Serum glutamic oxaloacetic transaminase levels rose in several patients. No other evidence of hepatic toxicity was seen. Objective remissions were noted in seven of 11 patients with cancer of the breast, two of five with melanoma, and two of six with cancer of the colon.

[Cancer Chemother Rep 56:413–419, 1972]

5-Azacytidine is an analog of cytidine in which nitrogen is substituted for the carbon in the 5 position of the pyrimidine ring. It was first synthesized by Piskala and Šorm in 1964. It is produced microbiologically by a species of *Streptovercillium*, *S. Ladakanus*. It is a white crystalline material which is quite soluble in water and various organic solvents. Its formula is $C_8H_{12}N_4O_5$ (1,2).

Studies with the compound reveal it to be active against several animal neoplasms, including the L1210 tumor (3,4). It was relative-

ly inactive, however, against the Walker 256 tumor. The compound was incorporated into plant and bacterial RNA and DNA and inhibited nucleic acid and protein synthesis (5,6). It is effective in causing chromosomal abnormalities in plants (7). Apparently, several patients have been treated in Europe; however, no published data on the results of the Czechoslovakian clinical studies have been available to date.

In animal experiments the efficacy of the drug as an antitumor agent was highly schedule-dependent, the most effective schedule being a daily dose given over 10 days.⁷ The maximum tolerated dose in the most sensitive animal, the beagle, was 0.55 mg/kg/day given over a 5-day period.⁷ Since data from animal studies seemed to indicate that a 10-day schedule would be proper for initial clinical use, patients in our study received beginning total daily doses of 0.03 mg/kg for 10 days.

MATERIALS AND METHODS

The drug was supplied in 50-mg vials. When the drug is to be administered, it is dissolved

⁷ Clinical brochure: 5-azacytidine (NSC-102816), Oct 1970, 25 pp. Prepared by the NCI, Bethesda, Md.

¹ Received Nov 17, 1971; revised Mar 17, 1972; accepted May 3, 1972.

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³ 5-Azacytidine: *s*-triazin-2(1*H*)-one, 4-amino-1- β -D-ribofuranosyl-. Supplied by the Cancer Therapy Evaluation Branch, Chemotherapy, NCI.

⁴ Performed as a phase I study for the Central Oncology Group (Dr. Robert O. Johnson, University of Wisconsin, Chairman).

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in an appropriate volume of distilled water and then given immediately. In this study, when the drug was given intravenously (iv), it was given as rapidly as possible. Two patients were treated at each dose level. A modified Fibonacci scheme was used to determine dose increments. After toxicity occurred, dose increments were limited to 10% of the previous dose. To be eligible for the study, patients were required to have a white blood cell count (wbc) of 5000 cells/mm³ or greater and a platelet count of 140,000 cells/mm³ or greater. The blood urea nitrogen (BUN) value had to be less than 12 mg/100 ml and the bilirubin value less than 1.2 mg/100 ml. Further contraindications for therapy included radiation to the pelvis, chemotherapy in the 4-week interval prior to therapy, prior adrenalectomy or hypophysectomy, or life expectancy of less than 3 months. Complete blood cell counts and platelet counts were done at least twice weekly and blood chemistry tests were done once weekly.

The criteria for objective remissions were those of the Central Oncology Group, that is, a 50% or greater reduction in the area of the tumors being measured as approximated by a 50% reduction in the product of the major

diameter of the tumor and its perpendicular. The regression must have lasted for a minimal period of 4 weeks with no new lesions appearing during this time. All lesions were measured weekly.

RESULTS

Tables 1 and 2 present a summary of the drug toxicity observed in the 30 patients treated. As can be seen, mild hematologic depression was noted at total doses of 12–16 mg/kg, and rather severe hematologic depression was noted in most patients receiving total doses of more than 16 mg/kg. The diarrhea noted was transient, occurring 2–3 hours after each injection; it was not disabling. In this series, we did not see stomatitis and dermatitis was rare. Hepatic function changes are found in animals treated with 5-azacytidine. Several of our patients had reversible rises in serum glutamic oxaloacetic transaminase (SGOT) levels, but no other hepatic abnormalities were noted. Although the hematologic depression was at times quite severe, serious infection occurred in only one patient and no patients died from this complication. Nausea almost invariably occurred after drug administration.

TABLE 1.—Hematologic toxicity

Patient No.	Sex	Dose*	Leukopenia			Thrombocytopenia		
			Nadir count ($\times 10^3$ cells/mm ³)	Day nadir occurred	Day count normal	Nadir count ($\times 10^3$ cells/mm ³)	Day nadir occurred	Day count normal
1	F	0.55 mg/kg, OD $\times$ 10	3.8	12	18	116	14	18
2	F	0.5 mg/kg, OD $\times$ 15	3.8	36	40	118	25	29
3	M	0.75 mg/kg, OD $\times$ 11	None	—	—	118	14	17
4	F	1.1 mg/kg, OD $\times$ 10	None	—	—	112	23	30
5	M	1.2 mg/kg, OD $\times$ 10	3.55	22	33	116	19	22
6	F	1.4 mg/kg, OD $\times$ 10	3.9	27	30	None	—	—
7	F	1.0 mg/kg, OD $\times$ 15	3.5	18	23	112	10	55
8	F	1.0 mg/kg, OD $\times$ 15	0.85	28	56	98	31	48
9	F	1.0 mg/kg, OD $\times$ 15	3.1	29	45	42	32	62

TABLE 1.—Hematologic toxicity—Continued

Patient No.	Sex	Dose*	Leukopenia			Thrombocytopenia		
			Nadir count ($\times 10^3$ cells/mm ³)	Day nadir occurred	Day count normal	Nadir count ($\times 10^3$ cells/mm ³)	Day nadir occurred	Day count normal
10	F	1.0 mg/kg, OD $\times$ 15	1.9	29	51	84	26	48
11	F	1.6 mg/kg, OD $\times$ 10	2.6	11	60	66	22	32
12	M	1.6 mg/kg, OD $\times$ 10	2.2	20	65	74	20	70
13	F	1.6 mg/kg, OD $\times$ 10	2.4	22	28	104	18	25
14	F	1.8 mg/kg, OD $\times$ 10	1.8	27	40	98	30	43
15	F	1.8 mg/kg, OD $\times$ 10	1.1	31	45	106	5	26
16	M	1.8 mg/kg, OD $\times$ 10	3.5	17	21	96	24	31
17	M	2.0 mg/kg, OD $\times$ 10	4.3	18	20	73	20	Died Day 20
18	M	2.0 mg/kg, OD $\times$ 10	0.5	18	Abnormal until Day 48	None	—	Died Day 48
19	F	2.0 mg/kg, OD $\times$ 10	None	—	—	88	49	56
20	F	2.0 mg/kg, OD $\times$ 10	1.2	22	42	48	28	42
21	F	2.0 mg/kg, OD $\times$ 10	0.95	23	72	46	20	55
22	F	2.0 mg/kg, OD $\times$ 10	2.9	32	37	90	11	77
23	F	2.0 mg/kg, OD $\times$ 10	3.3	23	68	96	54	86
24	M	2.0 mg/kg, OD $\times$ 10	1.0	28	50	52	31	53
25	M	2.2 mg/kg, OD $\times$ 10	0.9	29	39	32	17	27
26	F	2.2 mg/kg, OD $\times$ 10	0.25	23	57	38	23	54
27	F	2.4 mg/kg, OD $\times$ 10	1.0	26	50	116	26	28

*OD = daily dose.

Table 3 presents the initial remissions seen in this series and the relationship of dose to remission. Because parallel pharmacologic studies were being conducted (8), the patients selected for this study fell into relatively limited patterns of disease. Almost all patients treated who had either breast cancer or melanoma had soft tissue disease as their primary

manifestation of cancer. In the group of patients with breast cancer, most had either chest wall recurrences or unresectable tumors involving the breast, while most of the patients with melanoma had multiple, subcutaneous, metastatic disease. In either group, lesions were measurable. All objective remissions were partial; no complete remissions were seen.

TABLE 2.—Non-hematologic toxicity*

Patient No.	Sex	Dose†	Degree of—				Rise in SGOT value (units)	
			Nausea	Diarrhea	Stomatitis	Dermatitis	From	To
28	F	0.55 mg/kg, OD × 10	0	0	0	0		
1	F	0.55 mg/kg, OD × 10	3	2	1	0		
2	F	0.5 mg/kg, OD × 15	2	1	1	0	73.5	174
3	M	0.75 mg/kg, OD × 11	0	0	0	0		
4	F	1.1 mg/kg, OD × 10	2	1	0	0		
5	M	1.2 mg/kg, OD × 10	1	1	0	0	14.0	30.0
29	F	1.2 mg/kg, OD × 10	0	0	0	0		
30	F	1.2 mg/kg, OD × 11	0	0	0	0		
6	F	1.4 mg/kg, OD × 10	0	0	0	0		
7	F	1.0 mg/kg, OD × 15	2	0	0	0	16	40.0
8	F	1.0 mg/kg, OD × 15	3	1	0	0	40	168
9	F	1.0 mg/kg, OD × 15	2	1	0	0	26.5	86.5
10	F	1.0 mg/kg, OD × 15	2	2	1	1		
11	F	1.6 mg/kg, OD × 10	2	0	0	0		
12	M	1.6 mg/kg, OD × 10	1	1	0	0		
13	F	1.6 mg/kg, OD × 10	1	2	0	0		
14	F	1.8 mg/kg, OD × 10	2	1	0	0	20	95.0
15	F	1.8 mg/kg, OD × 10	1	0	0	0		
16	M	1.8 mg/kg, OD × 10	1	1	0	0		
17	M	2.0 mg/kg, OD × 10	2	0	0	0		
18	M	2.0 mg/kg, OD × 10	3	2	1	0	47.5	142
19	F	2.0 mg/kg, OD × 10	1	1	0	0		
20	F	2.0 mg/kg, OD × 10	2	1	1	1		
21	F	2.0 mg/kg, OD × 10	2	1	0	0	75	144
22	F	2.0 mg/kg, OD × 10	3	1	1	0	26.5	96.0
23	F	2.0 mg/kg, OD × 10	2	1	0	0		
24	M	2.0 mg/kg, OD × 10	2	1	0	0	12.5	29.5
25	M	2.2 mg/kg, OD × 10	2	2	0	0		
26	F	2.2 mg/kg, OD × 10	2	2	0	0		
27	F	2.4 mg/kg, OD × 10	2	1	0	0		

*0 = No toxicity; 1 = mild toxicity; 2 = moderate toxicity; 3 = incapacitating toxicity.

†OD = daily dose.

The data suggest that 5-azacytidine may be useful in the treatment of the soft tissue metastatic disease of breast cancer and malignant melanoma. One significant remission and one borderline remission were seen in patients with cancer of the colon.

Several of the patients with breast cancer who had objective remissions had received prior therapy with 5-fluorouracil (5-FU) or cytosine

arabinoside or both and were resistant to both.^a Thus, there did not appear to be any cross resistance to 5-azacytidine in patients treated unsuccessfully with other available antipyrimidines.

The length of the remission obtained is not

^a5-Fluorouracil: NSC-19893.

Cytosine arabinoside: NSC-63878; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride.

TABLE 3.—Response to treatment with 5-azacytidine

Disease	Patient No.	Sex	Treatment*	Response†‡
Cancer of the breast	1	F	0.55 mg/kg, OD × 10	S-O
	4	F	1.1 mg/kg, OD × 10	S-O
	6	F	1.4 mg/kg, OD × 10	O
	7	F	1.0 mg/kg, OD × 15	P
	9	F	1.0 mg/kg, OD × 15	P
	10	F	1.0 mg/kg, OD × 15	P
	16	F	1.8 mg/kg, OD × 10	S-O
	19	F	2.0 mg/kg, OD × 10	P
	22	F	2.0 mg/kg, OD × 10	S-O
	26	F	2.2 mg/kg, OD × 10	S-O
	27	F	2.4 mg/kg, OD × 10	S-O
Cancer of the colon	8	F	1.0 mg/kg, OD × 15	O
	16	M	1.8 mg/kg, OD × 10	P
	20	F	2.0 mg/kg, OD × 10	NC
	23	F	2.0 mg/kg, OD × 10	NC
	25	M	2.2 mg/kg, OD × 10	NC
	30	F	1.2 mg/kg, OD × 11	S-O
Melanoma	2	F	0.5 mg/kg, OD × 15	S-O
	5	M	1.2 mg/kg, OD × 10	S-O
	13	F	1.6 mg/kg, OD × 10	NC
	24	M	2.0 mg/kg, OD × 10	NC
	28	F	0.55 mg/kg, OD × 10	P
Cancer of the lung	3	M	0.75 mg/kg, OD × 11	P
	17	M	2.0 mg/kg, OD × 10	P
	18	M	2.0 mg/kg, OD × 10	P
Soft tissue sarcomas	12	M	1.6 mg/kg, OD × 10	P
	29	F	1.2 mg/kg, OD × 10	P
Cancer of the ovary	14	F	1.8 mg/kg, OD × 10	S-O
Cancer of the pancreas	21	F	2.0 mg/kg, OD × 10	S
Lymphoma	11	F	1.6 mg/kg, OD × 10	S-O

*OD = daily dose.

†P = progression of disease; S = subjective remission; O = objective remission; NC = no change.

‡Criteria for objective remission: (a) decrease of 50% or more in the product of the two perpendicular diameters of the lesions being measured (two consecutive measurements); (b) no deterioration of the patient's general condition; (c) no evidence of new lesions.

yet known since the drug has been used for only 10 months; however, two patients treated early in the study (one with carcinoma of the breast and one with melanoma) have remained in remission for 6 months.

Both of the long term responses have occurred in patients receiving maintenance therapy, the patient with breast cancer receiving an average dose of 150 mg of 5-azacytidine per week, and the patient with melanoma receiving an average dose of 225 mg per week. The initial remissions in patients not receiving maintenance treatment were almost invariably short, lasting an average of 6 weeks; we did not see any remissions lasting longer than 12 weeks in these patients. It was possible, however, to re-induce remissions in two patients with breast cancer who had relapses, one 8 weeks and the other 10 weeks after the cessation of therapy.

An attempt was made to establish a maintenance regimen for the patients who had had remissions. The minimum total weekly dose that produced moderate but tolerable hematologic toxicity was usually 2.4 mg/kg given twice weekly. This dose usually caused leukopenia with a total wbc of 2500–4000 cells/mm³ or thrombocytopenia with a platelet count of 80,000–110,000 cells/mm³ or both. There was significant variation, however, in tolerance to the drug of patients receiving maintenance therapy. The maintenance dose was commonly associated with moderate nausea; however, no patient refused to take the medication because of this.

DISCUSSION

5-Azacytidine, a pyrimidine analog, has been found to be quite active in a small group of patients, most of whom have breast cancer or melanoma. The data gathered to date present several interesting points.

Remission appears to be at least partially independent of dose in that remissions occurred at dose levels that gave no significant toxicity. Also, remissions commonly occurred early in the treatment course. Three patients with breast cancer had obvious shrinkage of their

tumors after 3 and 4 days of drug therapy and long before hematologic effects were noted, and a similar finding was noted in two of the patients with melanoma who had remissions.

It is apparent from the data obtained in animal studies that the total dose required to cause toxicity is significantly less when the drug is given intermittently over a prolonged period of time (ie, over 9 days) than when it is given as a short course of therapy.⁹ It is our impression that this may be so for man also since the total dose required to cause significant toxicity in man was 16 mg/kg when the drug was given over a 10-day course and approximately 10 mg/kg when given over a 15-day course.

Since the primary problem with the administration of drug, as far as the patient is concerned, is nausea, and since the severity of the nausea and vomiting appears to be directly related to the amount of drug given per injection, it is our feeling that patient tolerance will be much greater when the initial loading course is given over a prolonged period of time. Fortunately, this type of regimen appears to be the most effective in animals as far as anti-tumor activity is concerned, and our data to date, although extremely sketchy, lead us to believe that this will be the case for man as well.

It is also apparent that, compared with both dogs and rodents, man can tolerate high doses of the drug even when the doses are calculated on a milligram-per-kilogram basis. The reason for this is not clear since there are no obvious differences in overall patterns of excretion (8,9). The relative activities of kinases, deaminases, and other pertinent enzymes in various species are not known, however, and drug incorporation would be related to these. Also, it seems that the mechanism of action of the drug in man is not related to that of 5-FU or cytosine arabinoside and that resistance to one of these agents is not correlated with resistance to 5-azacytidine.

Initial observations in these patients appear to indicate that 5-azacytidine is promising as an anticancer agent in the treatment of breast cancer and melanoma. The series is obviously too small to enable us to make decisions concerning the drug's efficacy in other tumors.

⁹ See footnote 7.

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