A Phase I Study of 5-Azacytidine (NSC-102816)

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5-Azacytidine was first synthesized by Piskala and Sorm[1] in 1964, and their studies demonstrated that the drug was readily incorporated into both DNA and RNA. Its antineoplastic activity is attributed to its ability to inhibit DNA, RNA, and protein synthesis. This inhibitory action appears to be cell-cycle specific, and the greatest sensitivity is upon cells in the S-phase. Antitumor effect was demonstrated against L-1210 mouse leukemia with intermittent or continuous administration.

Studies with radioactive-labeled drug[3] indicated that absorption from subcutaneous injection sites was rapid, with peak plasma levels of radioactivity approximately equal to that noted in patients receiving the drug intravenously. The half-life after intravenous injection was 3.5 hours and after subcutaneous administration, 4.2 hours. Urinary excretion, however, was greater in patients receiving intravenous injections. The highest uptake of 5-azacytidine in tumor tissue was achieved when the drug was given intravenously. Small amounts of radioactivity were detected in the spinal fluid.

Phase I studies by Weiss and associates[2] and by Karon[4] indicated that the toxicity associated with the administration of this drug to humans was primarily nausea, vomiting, and diarrhea. These studies further indicated that these troublesome side effects appeared to be related to the amount of drug given in each injection. Hematologic toxicity consisted of granulocytopenia and thrombocytopenia usually occurring within three weeks after the start of chemotherapy. Transient elevations of SGOT were seen in some patients but there was no other evidence of hepatic toxicity.[5]

Weiss noted clinical responses in 7 of 11 patients with carcinoma of the breast, 2 of 5 patients with melanoma, and 2 of 6 patients with carcinoma of the colon. Subsequent studies with this drug in 29 patients with advanced gastrointestinal carcinoma revealed the anticipated toxicity with only one transient and partial objective response.[6] Tan and associates[7] studied 21 patients, 9 of whom were children, using intravenous 5-azacytidine. Leukopenia and thrombocytopenia occurred in all patients; nausea and vomiting was often severe but diminished with divided doses of the drug. Occasional ab-

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dominal pain, diarrhea, and fever were also noted. No regressions were seen in the patients with solid tumors of lymphomas in this series. One of 7 acute lymphatic leukemias had a poor remission. Karon et al.[4] studying 34 children with advanced acute leukemia receiving doses of 5-azacytidine ranging from 2 to 300 mg/m² daily X 5 noted responses in 6 of 12 myelocytic leukemias and 4 of 22 lymphocytic leukemia. Cunningham et al.,[8] using the agent in the treatment of carcinoma of the breast and comparing it with CCNU, noted a low response rate for both agents (two of 19 patients treated with CCNU and 2 of 21 patients treated with 5-azacytidine). Vogler et al.,[5] reporting on the phase I studies of the Southeastern Cancer Study Group, noted that 5-azacytidine given twice weekly at doses of 150–200 mg/m² would produce granulocytopenia after several weeks associated with nausea, vomiting, and diarrhea. No renal or hepatic toxicity was noted. Bellet et al.,[9] using the subcutaneous route of administration, noted only mild gastrointestinal toxicity at doses producing significant bone marrow depression. Hepatic toxicity occurred in five patients, possibly related to extensive hepatic involvement with metastatic tumor.

This report concerns the preliminary phase I study done by the Eastern Cooperative Oncology Group with this agent in patients with solid tumors.

Our study was undertaken as an attempt to determine the tolerated dose for the daily and once-weekly schedules of administration in the event that more definitive therapeutic studies were to be undertaken by the group.

Methods and Materials

Patients were selected for study who had progressive disease and who were not considered candidates for other group studies with a higher priority and for whom no other therapy was available. All had to be in reasonably good status and to have a life expectancy of at least three months. They were to have no evidence of bone marrow suppression and were to have been off all prior myelosuppressive or hormonal therapy and x-ray therapy for at least six weeks prior to entering the study. Cases were entered in a sequential pattern with only one patient at each of the cooperating institutions entering study per week for each schedule of drug administration. Four patients were to be entered at each level for each dose schedule during the initial phase of the study before proceeding to the next dose in the escalation pattern.

Participants could be withdrawn from the study at any time either upon request of the patient or the investigator if it was deemed in the best interest of the patient. No patient was to reenter the study after completing an initial drug trial unless eight weeks had elapsed from the time of the last drug dose.

Participants in the study were randomly assigned to either of the two groups. The first group received 5-azacytidine once weekly intravenously for a period of four weeks, and the second group received 5-azacytidine daily for five days followed by a nine-day observation period. A second course of five days was given after the observation period. At the end of four weeks, both treatment groups were observed for an additional four-week period for delayed toxicity or to allow toxicity to regress before being removed from the study.

The initial starting dose for the weekly schedule was 200 mg/m², and the starting dose for the five-day daily injection schedule was 50 mg/m². Incremental increases of both drugs were taken in a stepwise fashion as follows: step (1), increase by 100 per cent of starting dose; step (2), increase by 66⅔ per cent of starting dose; step (3), increase by 50 per cent of starting dose; step (4), increase by 33⅓ per

cent of the starting dose; and step (5), increase by 25 per cent of the starting dose. Weekly evaluations were made of drug toxicity and progress of the study by telephone conference calls among the participating investigators. No incremental increase or assignment of new patients to a new dose level was made without prior evaluation of the status of all patients actively participating in the drug study. Serial laboratory tests were performed according to the protocol design, and x-rays and measurements of lesions were made at two-week intervals where appropriate. The study was to be terminated when a tolerated dose without prohibitive toxic side effects was established for both the daily and weekly schedules of drug administration.

5-Azacytidine was reconstituted with 5 to 10 ml sterile water and administered by rapid intravenous push. After the first patients developed severe nausea and vomiting, chlorperazine (Compazine) was given parentally 15 minutes prior to the intravenous administration of 5-azacytidine. Chlorperazine by suppository was repeated during the next 24–48 hours as required.

TABLE I

Patient Distribution of Dose and Schelule of 5-Azacytidine

Dose (mg/m²)	No. of patients	No. of courses
	Daily Schedule	
50	4	7
100	3	6
133	3	5
158	2	3
Total	12	21
	Weekly Schedule	
200	4	14
400	4	15
533	5	15
633	2	5
Total	15	50

Results

Twelve patients received 21 courses of 5-azacytidine by the daily schedule and 15 patients received 50 weekly courses of drug by the weekly schedule (Table I). Table II shows the toxicity encountered at each of the dose levels for the daily schedule. Nausea and vomiting were the most prominent toxic side effects and occurred in every patient. Phenothiazines were only partially successful in relieving these symptoms. The vomiting usually had its onset 30 minutes to 2½ hours after the injection of 5-azacytidine and in some instances persisted for as long as 12–16 hours. In some patients, this was associated with diarrhea. As the severity of the nausea and vomiting increased, so did the degree of weight loss, and some patients refused to continue in the study after having severe nausea and vomiting during the first course of therapy.

Aside from the gastrointestinal toxicity (Tables II and III), we encountered no stomatitis and only one episode of skin rash. This was a transient mild maculopapular eruption which occurred during the fourth week of the weekly schedule of drug administration at a dose of 200 mg/m². One patient on the daily schedule and one patient on the weekly schedule showed a rise in blood urea nitrogen which returned to normal when the drug was discontinued. Two patients on the daily schedule also had a rise in uric acid from normal values prior to therapy to 9.6 and 10 mg/100 ml after completion of a five-day course of drug. (One of these patients also had both an increase in blood urea nitrogen and uric acid.) One possible instance of hepatotoxicity occurred in a patient receiving drug weekly as evidenced by a rise in SGOT from 30 units to

TABLE II

Daily Schedule Toxicity*

Patient	Carcinoma	Total 5-azacytidine dose (mg)	Wt. loss (kg)	Nausea	Vomiting	Diarrhea	Hematologic	Comments
				50 mg/m²				
O.S.	Endometrium	325	2	3	3	0	0	refused second 5-day course
P.M.	Lung	1200	0	2	2	0	0	
				1	1	0	2	Compazine
E.R.	Colon	720	0	2	2	1	0	
				1	1	1	0	Compazine
O.G.	CNS	700	0	1	1	0	0	
				0	0	0	2	Compazine
				100 mg/m²				
R.L.	Leiomyosarcoma	1500	1	2	2	2	0	
				2	2	2	0	
E.L.	Cervix	825	0	1	2	0	0	
				1	1	0	0	
J.G.	Lung	1500	2	2	2	0	0	
				0	0	0	0	Compazine
				133 mg/m²				
L.A.	Unknown	1575	3	1	2	0	0	
				1	1	1	0	Compazine
K.B.	Prostate	1000	1	2	2	0	4	rise in BUN (17-41 mg%)
J.M.	Colon	1125	3	2	1	0	1	rise in BUN (12-38 mg%)
				2	1	0	2	uric acid up to 9 mg%
				158 mg/m²				
W.S.	Lung	2000	2	2	2	1	3	
				2	2	2	4	
C.B.	Lung	2000	3	3	3	1	3	uric acid up to 10 mg%
					NO SECOND COURSE			

* Scale: 0, none; 1, mild; 2, moderate; 3, severe; 4, life threatening.

TABLE III

Weekly Schedule Toxicity*

Patient	Carcinoma	Total 5-azacytidine dose (mg)	Wt. loss (kg)	Nausea	Vomiting	Diarrhea	Hematologic	Comments
				200 mg/m²				
C.M.	Lymphosarcoma	1499	1	2	2	2	0	no control by Compazine, skin rash
P.M.	Larynx	1280	1	0	3	2	1	V & D after 4th inj.
J.M.	Melanoma	720	0	1	1	1	0	
B.D.	Melanoma	1400	0	1	1	1	0	
				400 mg/m²				
M.H.	Breast	1800	1	2	2	0	0	refused drug after 3rd dose
C.H.	CNS	1400	1	2	2	3	4	hematologic
M.R.	Prostate	2800	0	1	1	1	0	Compazine
M.J.	Tongue	2360	1	1	1	1	0	Compazine
				533 mg/m²				
J.C.	Stomach	800	1	3	3	2	0	refused to continue
J.C.	Cervix	1600	1	3	3	0	0	refused after 2nd injection
J.L.	Esophagus	3000	2	2	1	1	0	Compazine
J.A.	Unknown	3775	3	2	2	1	1	Compazine
C.B.	Lung	3100	2	2	2	1	1	Compazine
				633 mg/m²				
L.C.	Colon	3228	3	3	3	3	1	BUN rise (10-26 mg%)
R.B.	Kidney	3420	3	3	3	3	2	SGOT rise from 30-72 units

* Scale: 0, none; 1, mild; 2, moderate; 3, severe; 4, life threatening.

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