5-Azacytidine: A New Active Agent for the Treatment of Acute Leukemia

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Thirty-seven children with acute leukemia were treated with 5-azacytidine in 5-day courses given every 14 days. Six out of 14 children with acute myelogenous leukemia who were adequately treated achieved an M_1 marrow. Five of these subsequently developed complete remissions lasting 8 mo, 6 mo, 3 mo, 2 mo, and 2 mo. Of 22 children with acute lymphocytic leukemia, one achieved an M_1 marrow and one an M_2 marrow. The former attained a complete remission which lasted 3 mo. The maximum tolerated dose is between 150 to 200 mg/sq m on a daily x 5 schedule given every 14 days. The impressive activity of 5-aza-C in patients with acute myelogenous leukemia resistant to cytosine arabinoside indicates that this drug will become an important addition to the therapeutic armamentaria against this type of leukemia.

5-AZACYTIDINE (5-AZA-C) is an analog of cytidine first introduced for the treatment of acute leukemia of childhood in Czechoslovakia.[1] The critical step in the mechanism of drug action is not known, but the activity is presumably related to incorporation into DNA and RNA polynucleotides in place of cytidine.[2] The compound is known to interfere with DNA, RNA, and protein synthesis.[3,4] The drug shows preferential activity during the S phase of the cell cycle in vitro and has shown schedule dependency in animal systems.[5,6]

This study was undertaken to establish a tolerated dose of 5-aza-C on a daily × 5 schedule every 2 wk. During the course of this phase I investigation 5-aza-C was found to have impressive activity against acute myelogenous leukemia, and some activity against acute lymphocytic leukemia.

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Submitted January 22, 1973; revised March 12, 1973; accepted March 22, 1973.

These studies were performed under the auspices of Childrens Cancer Study Group A in the authors' institutions.

Supported by Grants CA-02649, CA-07306 and CA-07431 from the National Cancer Institute and Training Grant HD-00048 from the National Institute for Child Health and Human Development, NIH, DHEW.

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MATERIALS AND METHODS

Informed consent for the use of 5-aza-C was obtained from at least one of the parents of each child prior to treatment. This request was based on the potential efficacy of 5-aza-C and the fact that all other agents of known activity had been used. There were no parents who refused treatment for their child.

Thirty-seven patients ranging in age from 2 to 17 yr with acute leukemia refractory to standard chemotherapeutic agents were treated with 5-aza-C at a starting dose of 2 mg/sq m at the authors' institutions. The drug was administered as either an intravenous "push" or a 15-min "fast drip," daily for 5 days and repeated every 14 days, depending upon response and/or toxicity. Generally in the absence of significant toxicity, the dosage was increased by 50% increments (Fig. 1). In some instances the drug was administered in divided doses either every 8 or 12 hr to forestall nausea and vomiting.

Observations at the beginning of treatment included physical examination, a complete blood count, a bone marrow examination, platelet count, uric acid, SGOT, alkaline phosphatase, BUN, and urinalysis. During therapy, complete blood counts were repeated at least weekly and a bone marrow aspiration performed at least every 28 days. Criteria for evaluation were those in use by the Childrens Cancer Study Group A.[7] An M_1 marrow contains 5% or less of blasts or other abnormal cells.

5-aza-C was supplied in 50-mg vials by the Clinical Drug Evaluation Branch of the National Cancer Institute. The drug was dissolved in 5 ml of distilled water and administered within 15 min of reconstitution since the drug begins to decompose in aqueous solution within 1 hr.

RESULTS

Determination of Maximally Tolerated Dose

The pattern of dose escalation is illustrated in Fig. 1. Dosage increments were logarithmic below 100 mg/sq m. The rate of initial escalation was based on the lack of response and toxicity at the lower doses. The broken curve represents a modified Fibonachi numerical progression recently proposed by Dr. Oleg Sel-

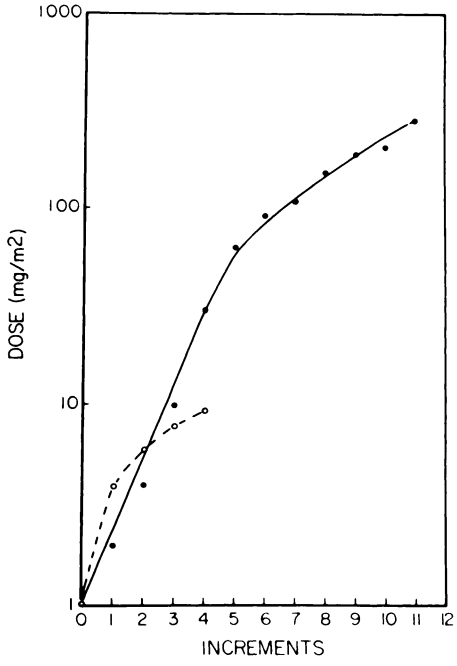

Fig. 1. Dose escalation of 5-azacytidine. Dose escalation was logarithmic until a dose of 100 mg/sq m when there was a decrease in this rate based on the occurrence of response and/or toxicity. Each point represents a dose increment actually used one or more times. The broken curve represents a modified Fibonachi search procedure designed to permit rapid escalation of the dosage initially while diminishing the rate of increase near the maximum tolerated dose in order to avoid excessive toxicity. The modified Fibonachi series is as follows: 2, 0.7, 0.5, 0.3.... These numbers are used as coefficients to predict dose increments. If the starting dose based on 0.1 LD_{10} in animals was 2 mg/sq m, then dosage increments would be 2, 4, 6.8, 10.2, 13.5 mg/sq m.... Blind adherence to such a scheme would unnecessarily prolong a phase I trial if the starting dose was too low.

awry for predicting dose increments.[7] The shape of this theoretical curve is quite similar to that which was obtained by increasing the dose until either response or toxicity occurred.

The maximum tolerated dose was obtained by determining the number of patients given a particular dose of 5-aza-C per course. As the dose of drug was increased, the number of patients at a given dose increased to a maximum and then declined abruptly between 150 and 200 mg/sq m. The plateau value of such a curve was used to estimate the lower limit of drug toleration.

Therapeutic Efficacy

The over-all results of treatment are tabulated in Table 1. Six out of 15 patients with acute myelogenous leukemia achieved an M_1 bone marrow status.

Five of these children obtained a complete remission which lasted 8 mo, 6 mo, 3 mo, 2 mo, 2 mo, and 2 mo. One patient with an M_1 marrow died within 3 wk in partial remission. The over-all remission rate for AML, therefore, is 6/15 (40%). If one excludes one patient with acute myelocytic leukemia who received only one-fiftieth of the maximum tolerated dose early in the study, then the remission rate becomes 6/14 (42%).

Of the 22 patients with acute lymphocytic leukemia (ALL), one patient achieved an M_1 marrow and another an M_2 marrow. The child with the M_1 marrow obtained a complete remission which lasted for 3 mo. The child with the M_2 marrow (9% lymphoblasts) died of infection before achieving a complete remission. The difference in remission rates (M_1) between AML and ALL is significant, $p < 0.008$ (Fishers exact test).

A summary of the clinical data on the eight patients who achieved an objective response is given in Table 2. Of these patients, six had white blood cell counts below 50,000 cells/cu mm at the time of diagnosis and two had a white blood cell count above 100,000 cells/cu mm. With the exception of 1 patient who received a lower dose of 5-aza-C 5 days each week, the best response occurred after two courses of drug in five patients and after four courses in two patients. At a dose of 150 to 200 mg/sq m per day × 5 the nadir in the white blood cell count occurred between the 10th and the 14th day.

There was no uniform approach to maintenance therapy for those patients who achieved complete remission. Patient CB, who stayed in remission for 8 mo, received 90 mg/sq m on 2 consecutive days each week. Patient DC (3 mo)

Table 1. Treatment Results

			Marrow			Over-all Status‡		
Diagnosis*	Patients	Adequate Treatment†	M-1	M-2	M-3	CR	PR	NR
AML	15	14	6	0	9	5	1	9
ALL	22	22	1	1	20	1	1	20
Total	37	36	7	1	29	6	2	29

*AML, acute myelogenous leukemia as a generic name for all subcategories of non-ALL; ALL, acute lymphocytic leukemia, including acute undifferentiated leukemia, AUL.

†Adequate treatment is at least one course at a dose of 60 mg/sq m or greater daily x 5. The one inadequately treated patient had less than 10 mg/sq m daily x 5.

‡CR, complete remission, PR, partial remission, NR, no response or progressive disease.

Table 2. Clinical Characteristics of Responding Patients

Patients	Age/Sex	Diagnosis*	Previoust Treatment	White Blood Cell Count x 10^3			Dosage (mg/sq m)‡ in Induction			Duration of Induction Phase		Response		
				Diagnosis	Start of Treatment with 5-aza-C	Nadir	Initial	Final	Total	Wks	Courses	Marrow	Over-all Status	Duration
C.B.	14 F	AML	VCR, ara-C CTX, MP	113	3.4	0.9	2	90	1597	15	8	M-1	CR	3 mo
D.C.	3 M	AMML	ara-C, VCR, MP	3.9	5.9	0.4	140	160	1180	5	2	M-1	CR	3 mo
J.B.	5 M	ALL	VCR, MTX Asp ara-C, MP DBD	174	16.6	0.6	80	150	2431	9	4	M-1	CR	3 mo
N.S.	7 F	AMML	VCR, ara-C, CTX TG, P VCR, ara-C	25.7	2.0	0.6	125	268	3411	8	4	M-1	CR	6 mo
R.R.	5 M	AMML	VCR, ara-C CTX, DNM	21.6	4.3	0.6	93	93	1395	6	2	M-1	PR	3 wks
C.K.	8 M	ALL	P, VCR, MTX, Asp	21.5	0.7	0.7	166	222	1944	4	2	M-2	PR	3 wks
K.K.	2 F	AML	VCR, P, ara-C, CTX, TG MP	20.2	10.9	4.0	150	150	1500	4	2	M-1	CR	4 mo
P.J.	10 M	AML	VCR, CTX ara-C, TG P, MP	15.6	1.0	0.7	150	150	1500	4	2	M-1	CR	2 mo

*AML, acute myelocytic leukemia; AMML, acute mono-myelocytic leukemia; ALL, acute lymphocytic leukemia.

† VCR, vincristine; ara-C, cytosine arabinoside; MP, 6-mercaptopurine; MTX, methotrexate; CTX, cyclophosphamide; ASP, L-asparaginase; P, prednisone; TG, thioguanine; DBD, dibromodulcitol, DNM, daunomycin.

‡ Daily x 5 every 14 days, except for CB (see text).

received 150 mg/sq m q.d. for 2 consecutive days every week. Patient NS is receiving 230 mg/sq m q.d. × 5 every 3 wk. Patient JB has received 150 mg/sq m q.d. × 5 every 5–6 wk. This more prolonged interval was necessary because JB's white blood cell count continued to decrease for approximately 4 wk following therapy. Patients KK and PJ were maintained on 5-aza-C 100 mg/sq m q.d. × 5 once per month.

Toxicity

The most disabling toxicity involves the gastrointestinal tract. At doses of 150 mg/sq m or above, 5-aza-C causes profound nausea and vomiting and diarrhea in all patients. The severity of these toxic manifestations, especially the former, can be reduced by giving the drug in divided doses or in some instances by the use of a 15 min intravenous fast drip. Antiemetics such as chlorpromazine can be of use when given in relatively large doses at least 24–48 hr prior to beginning the course of 5-aza-C. A pruritic, follicular skin rash occurred in 50% of the patients, but was usually transient and did not require drug dosage modification.

The drug is myelosuppressive. Although recovery usually occurs by day 14 at a dose of 150 mg/ sq m daily × 5, myelosuppression may be more prolonged, especially at 200 mg/sq m or above. Myelosuppression is not necessarily untoward and accompanied response in every instance except one (Table 2).

DISCUSSION

These data indicate that 5-aza-C is an active drug for the treatment of acute leukemia in children. The compound has particularly impressive activity against acute myelogenous leukemia. Activity of 5-aza-C against adult leukemia has recently been demonstrated by McCredie and co-workers[8] and against acute lymphocytic leukemia in newly diagnosed patients who were also receiving prednisone.[1]

The fact that the activity of 5-aza-C could be demonstrated in children with advanced refractory leukemia has important implications for the evaluation of other new drugs. Agents of uncertain potency do not need to be tested early in the course of a child's disease to demonstrate activity. Indeed, the activity of vincristine, ara-C, and L-asparaginase, all effective antileukemic agents, was demonstrated under similar circumstances. Since 5-aza-C is not cross-resistant to ara-C, the use of these two agents in combination for the treatment of AML should be promising.

Although the original plan was to adhere to a modified Fibonachi search for dose escalation, this approach was abandoned. The Fibonachi search involves a rapid initial dose escalation followed by decreasing dosage increments which are predetermined and aimed at reducing the chance of grossly overshooting the maximum tolerated dose. The original plan for escalation is shown on Fig. 1 as an interrupted line, and is based on the series 2, 0.7, 0.5, 0.3.... The initial starting dose was 2 mg/M^2, $\frac{1}{10}$ the LD_{10} determined in preclinical pharmacology. Because this dose proved to be 70–100-fold less than the maximum tolerated dose, strict adherence to Fibonachi's escalation program would

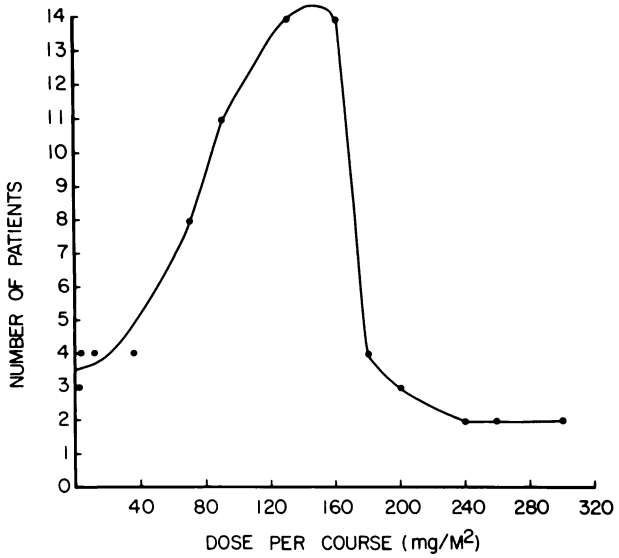

Fig. 2. Frequency distribution of patients receiving a particular dose of 5-aza-C per course. The plateau of this curve determines the maximum tolerated dose of 5-aza-C since courses which do not produce toxicity or therapeutic effect or which produce excessive toxicity are usually not given to a large number of patients, while tolerated courses that affect the disease favorably are used in an increasing number of patient. All patients receiving the drug are included as long as they were in the induction phase. A patient can be represented only once for each dose level.

have been completely unworkable. The shape of the actual dose escalation curve, however, is similar to the Fibonachi modification, indicating that there is a need to reduce the rate of dosage increment once there is either a therapeutic effect or some toxic manifestations, but that this mathematical series per se has no particular "magic."

The maximum tolerated dose was estimated by plotting the number of patients receiving a given dose per course. Clearly such a curve would have a positive slope at doses which were well tolerated and, therefore, used in more patients and a negative slope when the tolerated dose was exceeded. This proved to be the case (Fig. 2) and was a useful way of predicting drug dosage. The main danger of a more rapid escalation scheme is the development of cumulative toxicity. This can be obviated by escalating the dosage every second course as the maximum tolerated dose is approached.

The achievement of complete remission in two patients whose initial white counts were in excess of 100,000 indicates that such high white blood cell counts may not necessarily predict for a poor prognosis, especially in advanced disease. This may be the result of a selection since patients with acute leukemia who live long enough to receive phase I agents have usually responded to other agents. The practice in some phase II studies designed to estimate the remission rate in advanced disease to exclude certain patients because of the height of their initial white blood cell count may not necessarily be justified.

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