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EFFECTS OF 6-AZAURIDINE ON REGENERATING BONE MARROW*

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Abstract—The pyrimidine nucleoside antimetabolite, 6-azauridine (AzUR), was studied for its effect on the regeneration of bone marrow in heavily irradiated mice with marrow repopulated by transfusion of isologous marrow cells. AzUR, a drug that has antitumor activity, but relatively little toxicity for normal bone marrow in mice, suppressed marrow regeneration when given at 8-hr intervals for a week in total daily doses of 10–620 mg/kg, or when administered in a single large dose, 1,000 or 3,000 mg/kg, on the fourth day after irradiation and seeding with isologous marrow cells—a tme when the cells appear particularly sensitive to cytotoxic agents. These observations indicate that the marrow precursor cells behave in a manner resembling that of certain types of neoplastic cells in their responsiveness to drugs, and support the earlier suggestion that the regenerating marrow system can be of value in detecting drugs with antitumor potential.

PRIMITIVE stem cells that appear in the bone marrow of heavily irradiated mice after the intravenous injection of isologous bone marrow have been shown to be unusually susceptible to cytoxic drugs. After irradiation and treatment with isologous marrow, cytotoxic agents, in amounts much smaller than those required to suppress normal bone marrow function, decrease the rate of hematopoietic regeneration. This sensitivity to drugs is most marked 3–5 days after irradiation and intravenous administration of the seeding isologous marrow, with a resultant total femoral marrow cell count of approximately 300,000, in contrast to a normal value of about 10–20 million marrow cells. At this time, these cells of the regenerating marrow consist of large blast forms and a few small lymphocytic cells, with almost no cells recognizable as hematopoietic precursors. Once the logarithmic phase of marrow regeneration has been reached, however (usually 6–8 days after irradiation and marrow infusion), this sensitivity to cytotoxic drugs is lost.[1]

It will be apparent that the behavior of these primitive hematopoietic precursor cells, with respect to their response to cytotoxic drugs, is similar to that of tumor cells. Unlike tumor cells, however, these marrow blasts seem generally responsive to almost all cytotoxic agents without the partial selectivity that may be seen with some drugs for certain types of neoplasms.

One of the newer pyrimidine nucleoside analogs, 6-azauridine (AzUR), exhibits certain biologic properties that make it of interest in the study of the marrow regeneration system. This drug is virtually nontoxic in normal mice; thus, 75 mg/kg, in three

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divided parenteral doses daily for 6 or 7 days, causes little or no weight loss. In such doses, however, AzUR inhibits the rate of growth of several transplanted mouse tumors.[2, 3] In humans, the drug appears to be without toxicity for normal marrow, although it exerts definite inhibitory action in certain acute leukemias, particularly those of the monocytic variety.[2, 4] Because of its specificity for the leukemia cell, with little toxicity for normal tissues, AzUR approaches in principle the ideal type of compound for cancer chemotherapy. Unfortunately, however, tolerance (not true drug resistance) appears, and the effect on susceptible neoplastic cells is short-lived.[5, 6] Because of this differential effect between normal tissues and tumors, it was of interest to determine whether the stem cell of the regenerating marrow is responsive to AzUR.

MATERIALS AND METHODS

All experiments were performed in (C3H × C57) F_1 mice, aged 10 to 15 weeks.* These hybrid mice are quite hardy and were particularly useful in this work because their femoral marrow counts are normally greater than those of other strains used in this laboratory in previous experiments.

Femoral marrow counts were performed as previously described.[1] The animals were sacrificed and their femurs removed. In the experiments, 4 to 6 mice comprised each experimental group; the femurs of these animals were put into duplicate pools, the cells were counted separately, and an average femoral marrow count was determined. In preparing the marrows, the neck of each femur was severed, and 1·0 ml of saline in a syringe with a 23-gauge needle was used to flush the marrow from the bone into a small beaker for collecting the pool of marrow cells for counting. Leukocyte counts were performed in duplicate in the usual manner; in later experiments a Coulter electronic particle counter was used. Statistical analysis of results of the counts obtained in 25 duplicate pools with 'high' (above 10,000,000) and 'low' (below 5,000,000) values revealed similar variations of the duplicate pools of 13 and 15% respectively. Thus, the total error in both preparing the marrow pools and performing the cell counts is within 15% of the mean—an acceptable error in such a biologic system.

During the studies, animals were housed in groups of 6 or less per cage. Because in most experiments different daily injections were given to five or more groups of 4–6 mice each, it was not feasible to house the groups randomly. Some variation in results was seen occasionally in animals in one group; this may have been related to unapparent infection or to other events arising within that cage.

Irradiation was given as previously described;[1] 800 r in these experiments was uniformly lethal to untreated mice. In all experiments, approximately 10,000,000 nucleated isologous cells in 0·5 ml of Hanks' solution were administered intravenously soon after irradiation. In most experiments the animals were sacrificed for femoral counts 7 days after irradiation, since it had been established that an inoculum of 10,000,000 cells caused within 7 days a regeneration of marrow counts to about 50% of the normal level in animals not treated with cytotoxic agents. Drugs were prepared daily for each experiment in sterile pyrogen-free water; administration was by the intraperitoneal route, in volume of 0·1 m/dose. Sterile pyrogen-free saline was administered to the controls.

* Supplied by Cumberland View Farms, Cumberland, Tenn.

RESULTS

A. Effects of 6-azauridine in normal mice

Although the data of Welch and his associates[2] had indicated that AzUR was almost nontoxic at 75 mg/kg, given in three divided intraperitoneal doses daily, we studied a group of normal mice injected intraperitoneally with even larger doses, up to as high as 500 mg/kg, given as a single daily dose for one week. At the highest dose level, the animals appeared ill, lost weight, and exhibited some depression of cellular activity in the bone marrow (Table 1).

TABLE 1. EFFECTS OF 6-AZAURIDINE (AzUR) ON FEMORAL BONE MARROW TOTAL CELL COUNT IN NORMAL MICE

Daily dosage of AzUR, i.p. (mg/kg)	Marrow count on day 7 (millions)
0	25·0
125	29·8
250	24·9
500	17·3

B. Effect of daily injections of 6-azauridine on marrow regeneration

In a preliminary experiment, doses of 7·5 to 25 mg AzUR/kg appeared to inhibit regeneration in a linear, dose-related manner similar to that previously described for other drugs.[1] Repeated attempts to confirm that observation, however, have failed to produce a similar result, and the preliminary observation cannot be explained. In a large number of experiments daily doses of AzUR of 25–200 mg/kg inhibited regeneration modestly, but the depression of the rate of cellular proliferation never exceeded 50% of the control values. The results were somewhat erratic and inhibitions definitely were not linearly related to dose even though the dosages approached those that are toxic for normal animals. Uridine, the normal metabolite, was clearly not inhibitory in similar or even larger daily dosage. Uridine given 0·5–2 hr before the antimetabolite did not seem to diminish the effect of AzUR.

C. Effects of 6-azauridine at 8-hr intervals

Because of the irregular results obtained with daily AzUR, a more frequent dosage schedule was employed. Welch and Jaffe, and their associates,[2, 3] had demonstrated antitumor effect only when AzUR was administered in divided daily doses, and Pasternak and Handschumacher[7] reported rapid renal excretion of AzUR in mice. Administration of AzUR in amounts of 10–620 mg/kg daily in 3 divided doses (every 8 hr) for 7 days produced dose-related inhibition of cellular regeneration; two experiments are illustrated in Fig. 1. Dosage beyond 160 mg/kg failed to reduce further the already striking depression of marrow replacement, and the animals tolerated these large doses without gross toxicity or mortality.

D. Effects of a single 6-azauridine dose given 4 days after irradiation

Since studies with other cytotoxic agents had demonstrated that a single dose of drug, given 3–5 days after irradiation and repopulation, inhibited marrow regeneration, this effect was investigated with AzUR. Larger doses than those studied in the regimens of daily dosages were employed, with a single intraperitoneal dose in 0·1-ml volume

given 4 days after irradiation; the marrow counts were done on day 7 in the usual manner.

In this system a very definite response to AzUR was observed, and this was dose-related (Table 2); the effect of the drug was reproducible, with 1,000 mg/kg giving about 50% inhibition. A dose of 3,000 mg/kg in another experiment caused 78% inhibition on day 7 and 40% on day 8.

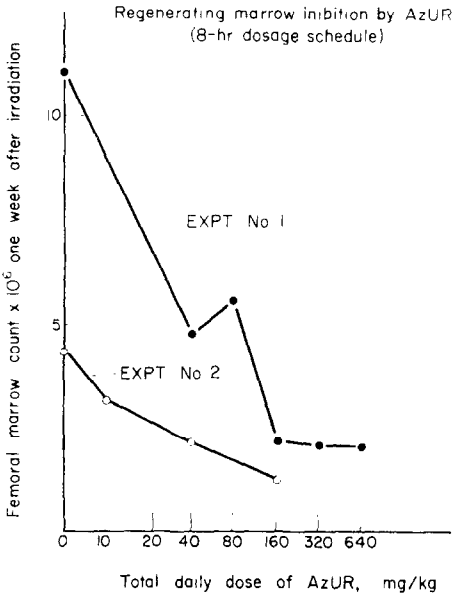

FIG. 1. Results of two experiments in irradiated, marrow-treated mice given AzUR at 8-hr intervals. AzUR produced dose-related marrow inhibition, but a maximal effect was attained with total daily dosage of 160 mg/kg.

Blocking the effect with the normal metabolite, uridine, was attempted by giving the normal metabolite 0·5 hr before the antimetabolite (Table 3). The uridine in these large doses was moderately inhibitory in itself and appeared to enhance the toxicity

TABLE 2. EFFECT OF A SINGLE DOSE OF 6-AZAURIDINE GIVEN 4 DAYS AFTER IRRADIATION AND MARROW REPOPULATION

Single dose of AzUR (mg/kg)	Marrow count on day 7 (millions)
0	10·6
1,000	5·3
2,000	3·0
3,000	1·9

of AzUR; indeed, the last combination tested (see Table 3) killed most of the animals. Further observations indicated that doses of uridine above 1,000 mg/kg inhibited regeneration in nonlinear fashion, producing 20–40% inhibition in most experiments and killing occasional animals in doses above 5,000 mg/kg. It was considered that this

result might be attributable to the osomotic effects of the large amount of uridine dissolved in only 0·1 ml of water. Dilution of the uridine in 0·5 ml of water possibly caused somewhat less inhibition of the marrow but still was lethal to some animals in the high dose range. Three experiments in which 1,000–2,000 mg uridine/kg was used

TABLE 3. EFFECT OF SINGLE DOSES OF URIDINE, 6-AZAURIDINE, OR COMBINATIONS ON MARROW REGENERATION

Material injected i.p. on day 4	Marrow count on day 7 (millions)
Saline	11·7
AzUR (3,000 mg/kg)	3·1
Uridine (5,000 mg/kg)	7·8
Uridine (5,000 mg/kg + AzUR 3,000 mg/kg)	2·1
Uridine (10,000 mg/kg + AzUR 3,000 mg/kg)	1·3

in an attempt to block the effect of 1,000 mg AzUR/kg were inconclusive with respect to either protective or additive actions.

DISCUSSION

The pyrimidine nucleoside antagonist, 6-azauridine (AzUR), inhibits the regenerating bone marrow of the mouse in dose-related fashion, and the response occurs with amounts of drug that produce no toxicity in normal mice. Retardation of marrow repopulation after irradiation and isologous marrow transfusion was demonstrable when relatively high levels of AzUR were maintained by administration of modest doses of the drug at 8 hr intervals for a week, or when a single very large amount of drug was given on the fourth day after irradiation and repopulation. Under these conditions only a relatively small number of primitive cells constitutes the hemotopoietic potential of the animal. Daily drug administration at intermediate dose levels caused inconstant interference with marrow regeneration, probably because the renal excretion of the drug is so rapid that the opportunity for inhibitory action was quite transitory.

The demonstration that AzUR does inhibit the regenerating mouse bone marrow is of particular interest because this compound is so extraordinarily benign to normal human and mouse marrow. In our test system, the marrow is first obliterated with irradiation and then repopulated by intravenously administered isologous marrow cells; the few cells that are responsible for the rapid regeneration behave more like tumor cells than normal marrow constituents in their responsiveness to AzUR. Because these precursor cells are capable of such extremely rapid cellular proliferation, one is inclined to attribute their drug sensitivity to their metabolic activity. Although this assumption is logical, two other mechanisms must be considered: (1) the repopulating cells must migrate to the marrow cavity, and their lack of firm tissue fixation might in some way enhance their drug sensitivity; and (2) after the large dose of X-radiation necessary to destroy autologous marrow, perhaps few cells, except the intravenously administered marrow precursors, are able to undergo mitosis. This

might, in effect, provide a host in which growth-inhibiting compounds were selectively concentrated in a few proliferating cells. We have been unable to devise experiments that would more clearly define the mechanism of sensitivity of the regenerating marrow to cytotoxic agents. These studies with AzUR, however, provide further evidence of the value of this test system in detecting compounds with potential antitumor activity.

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