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Some Effects of Chemotherapeutic Drugs on Bone Marrow Stem Cells

I. The Long-Term Effects of Phase-Specific Drugs on Mouse Bone Marrow Stem Cells

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Summary. *Two phase-specific drugs, cytosine arabinoside and hydroxyurea, were studied with regard to their effects on various murine hematologic cell compartments of the same mouse.*

Effects of single and multiple injections of Ara-C were compared.

Following a significant decrease in the first few days, and a subsequent overshoot of pluripotential stem cells (CFU-S), colony-forming cells (CFC), bone marrow nucleated cells, and leukocytes, the number of these cells returned to normal values with a time sequence that varied with the cell type. During the 6-month observation period the number of these cells oscillated around control values after both drugs and both types of protocols.

Introduction

Several studies have investigated the survival curves obtained for different murine hematopoietic bone marrow cells subjected to chemotherapeutic drugs [4, 7, 2, 10, 6, 15]. Data on the repopulation of stem cells after administration of various drugs have been reported, but without systematic comparison between the different maturation compartments of the bone marrow [16, 5, 3, 14, 12, 11]. Moreover, all these studies were short-term, not exceeding 20 days in length.

The aim of this paper is to compare the effects of drugs on stem cells and the different hematologic compartments of the granulocytic series in the same mouse at short-term intervals and up to 6 months after treatment to assess any residual damage. We studied two phase-specific drugs: cytosine arabinoside (Ara C) and hydroxyurea (HU), and determined the numbers of CFU-S, pluripotential stem cells (studied by the spleen colony technique), CFC, unipotential bone marrow cells committed to differentiation towards the formation of granulocytic-macrophage colonies, bone marrow nucleated cells, and white blood cells (WBC) to elucidate the mechanisms for the repopulation of hematopoietic cells. These studies were also carried out after fractionated doses of Ara-C, to compare the effects with those of single doses. Correlations between CFUs and CFC were carried out to determine whether CFC reflect the behavior of CFU-S in view of clinical interpretation where only CFC can be assessed.

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Materials and Methods

Mice

This study was done on 507 female CBA/OLA SPF mice between 2 and 3 months of age. Their average weight was 20 mg. Each experimental group consisted of three donor mice and ten recipient mice.

Drug Treatment

On day 0, we injected three different donor groups:

a) 30 mg AraC (Upjohn),
b) 3 × 10 mg AraC (one injection every 4 h), or
c) 5 mg HU (Squibb).

On days 1, 2, 3, 4, 5, 8, 10, 16, 22, and 30 and at 2, 3, and 6 months after the above injections, we assessed the numbers of CFUs, CFC, and nucleated cells in the bone marrow of one leg and the number of WBC per cubic millimeter of blood.

Evaluation of CFU-S (colony forming units)

The number of CFU-S present in the bone marrow was determined by the spleen colony method of Till and McCulloch [13]. The bone marrow contained in one femur and one tibia from each of three mice was flushed out with NCTC-199 medium and single cell suspensions were obtained by flushing with syringe and needle. An ap-

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propriate dilution was made for IV injection of 8.10^4 cells in 0.2 ml to mice previously given 900 rads of whole-body ^{137}Cs irradiation. Nine days later the mice were killed, and their spleens were removed and fixed in Bouin's solution. Macroscopic spleen colonies were scored 24 h later.

Evaluation of CFC

The number of CFC was determined by the in vitro technique of Worton et al. [19]. We cultured 10^5 bone marrow cells in α medium containing serum, methyl cellulose, and colony-stimulating factor (CSF) obtained from the serum of C_{57} Bl_6 endotoxin-injected mice. After 7 days of incubation at 37° C in high humidity the numbers of granulocytic-macrophage colonies were scored with an inverted microscope.

Evaluation of the Percentage of CFU-S in S Phase

The suicide technique of Becker et al. [1], with high-specific-activity ^3H-TdR was utilized to assess the percentage of CFU-S in DNA synthesis in the mice that received three injections of Ara C. This study was not done in the other protocols.

Results

Colony-Forming Units

Twenty-four hours after injection of a single dose of 30 mg Ara C, the number of CFU-S decreased to 47%

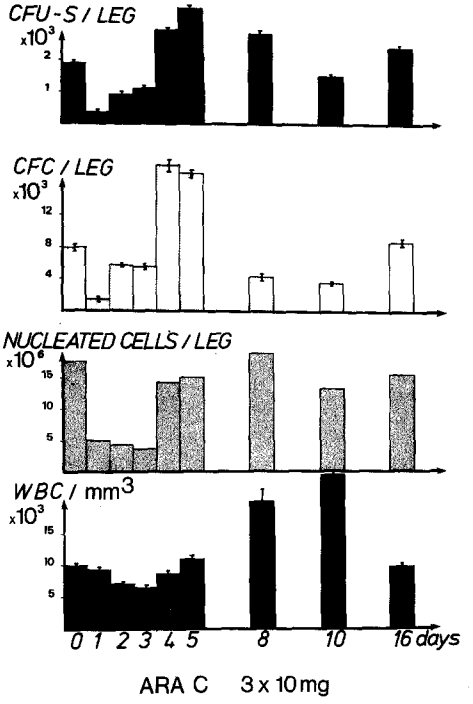

Fig. 2. Time course changes in (top to bottom) the number of CFU-S, CFC, and nucleated cells per leg, and in the number of white cells per cubic millimeter, after injection of 3 × 10 mg Ara C

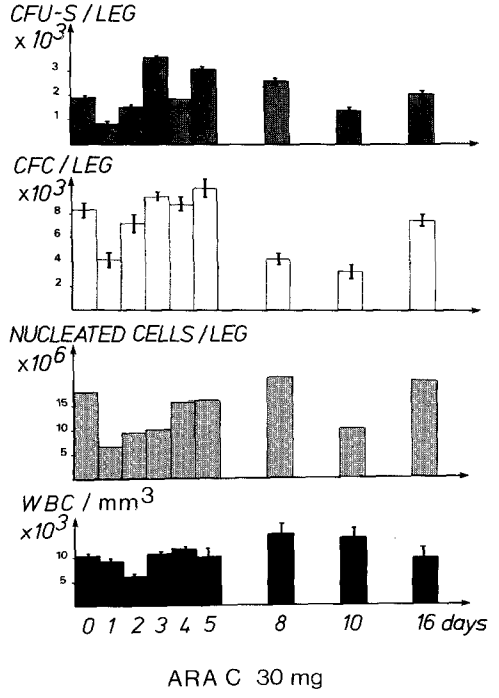

Fig. 1. Time course changes in (top to bottom) the number of CFU-S, CFC, and nucleated cells per leg, and in the number of white cells per cubic millimeter, after injection of 30 mg Ara C on day 0

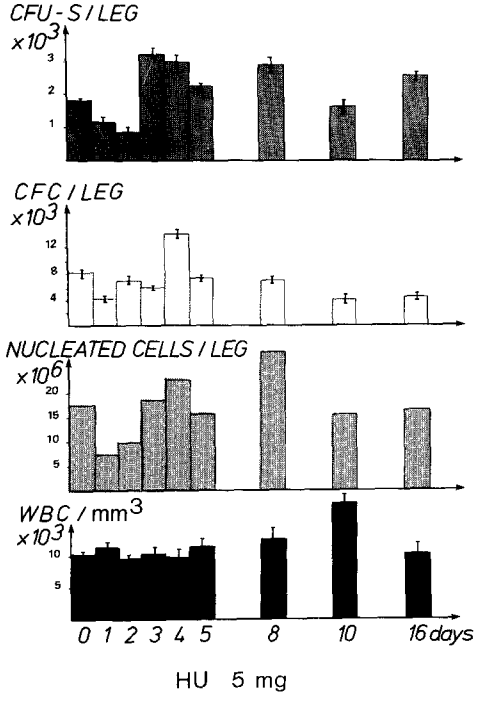

Fig. 3. Time course changes in (top to bottom) the number of CFU-S, CFC, and nucleated cells per leg, and in the number of white cells per cubic millimeter, after injection of 5 mg of HU

and to 77% of the control values when expressed per total number of nucleated cells per leg and per 10^5 cells, respectively. Twenty-four hours after three doses of 10 mg Ara C by injection a more pronounced effect was observed: 20% and 45% of control values when calculated per leg and per 10^5 nucleated cells, respectively (Figs. 1 and 2).

Three days after the single 30 mg Ara C injection, and 5 days after the last of three separate 10-mg Ara C injections, the number of CFU-S per leg was 190% and 200% of control values, respectively. This overshoot was followed by a return to normal values after both protocols by 8–10 days after the injections.

Twenty-four hours after 5 mg HU the number of CFU-S decreased to 65% and 96% of the control CFU-S values when expressed per leg and per 10^5, respectively. The decrease continued, so that by 48 h after injection the CFU-S level was 41% (per leg) and 46% (per 10^5 cells) of the control values.

Sixteen days after both single and multiple Ara C and single HU treatments, the number of CFU-S was close to normal values and remained constant for 2 months. There seemed to be a slight nonsignificant overshoot at 3 and 6 months after 3 × 10 mg Ara C.

Colony-Forming Cells

Twenty-four hours after the injection of 30 mg Ara C, the number of CFC per leg was 41%, and after 3 × 10 mg it was 15% of the control value. After 5 mg HU the number of CFC per leg decreased to 60%.

The number of CFC per 10^5 nucleated cells was 105% of the control value after 30 mg AraC, 55% after 3 × 10 mg AraC, and 94% after 5 mg HU.

Sixteen days after HU and AraC treatments, the number of CFC was close to normal values, and it remained constant for 6 months.

Percentage of CFU-S in DNA Synthesis (S Phase)

In the controls, 8% of CFU-S were in DNA synthesis. Four hours after the first injection of 10 mg Ara C, 11.5% of the CFU-S were in DNA synthesis and 4 h after the second injection 31% of the CFU-S were in S, as shown in Table 1.

Bone Marrow Nucleated Cells

A decrease in the number of nucleated cells per leg was evident 24 h after the injection of either drug: $6–7.10^6$ cells per leg, compared with 17.10^6 for the controls. The number of nucleated cells then returned to control levels by the fourth day and remained constant throughout the observation period of 6 months.

White Blood Cells

After one injection of Ara C, the number of circulating leukocytes decreased from 10,000 to 6,000 per cubic millimeter in the first 2 days. On the eighth and tenth days there was an increase in the leukocyte count to a higher level than that of the controls. This was then followed by a return to normal values at 16 days after treatment.

The minimum WBC level (6,000 WBC/mm^3) was obtained on the second and third days after three injections of 10 mg Ara C. The overshoot here was greater than after a single dose, and at 10 days the number of WBC reached 25,000/mm^3.

After HU treatment, the number of leukocytes was not different from the controls up to the tenth day, when there was an increase to 18,000/mm^3.

In all protocols, the number of WBC was back to normal 1 week after treatment and remained normal during the observation period of 6 months.

Table 1. Number of CFU-S, CFC and nucleated cells per 10^5 and per femur 4 h after the first injection of 10 mg Ara C and 4 h after the second injection of 10 mg Ara C, and evaluation of the percent age of CFU-S in DNA synthesis at the same moments

	CFU-S		% in S phase	CFC		Nucleated cells per femur
	per 10^5 and as % of controls	per femur and as % of controls		per 10^5 and as % of controls	per femur and as % of controls	
Controls	19.8 ± 2.3	1,287 ± 143	8	78.5 ± 1.5	5,102 ± 97	6.5 10^6
4 h after 10 mg Ara C (time of second injection)	16.8 ± 1.2 85%	941 ± 67 73%	11.5	56 ± 3 71.3%	3,136 ± 168 61.5%	5.6 10^6
4 h after 2nd injection of Ara C (time of third injection)	20.7 ± 3.1 104%	1,262 ± 192 98%	31	43 ± 4.5 54.7%	2,623 ± 274 51.4%	6.1 10^6

Discussion

Better understanding of the damage to stem cells and of the repopulation of the stem cell compartments may help to avoid hematologic complications during cancer treatment. It seems of major interest to monitor the early events for the acute consequences of chemotherapy, and late events for residual injury and secondary leukemia.

This paper presents some data on the bone marrow of mice, correlating the kinetics of the various hemopoietic compartments of this tissue and comparing the effects of different protocols. Such a study is not possible as yet in patients.

All these studies were carried out in the bone marrow and will be completed by studies in the spleen.

The first observation to point out in this paper is that 24 h after one or three injections of Ara C or HU, the CFU-S, CFC, and nucleated cell compartments are depleted. This depletion was more significant after the 3 × 10 mg Ara-C protocol than after a single 30-mg injection. The decrease in the WBC counts became evident much later (3 or 4 days) after both protocols of Ara C treatment and no decrease was observed after HU.

The depletion of the CFC compartment is due to the direct action of the drugs used, since they are S phase-specific and about 50% of CFC are in S phase in the normal mouse. The depletion of the CFU-S compartment is not due to the direct effect of the drugs, since CFU-S are quiescent in normal mice, as previously found after HU and ^{3}H-TdR [18]. Previous unpublished results have shown that the CFU-S compartment is depleted later than the CFC compartment after injection of one dose of Ara C, and this can best be explained by a differentiation process of the CFU-S as a consequence of the depletion of the more mature compartments. As for the nucleated cells that are in cycle, they are vulnerable to the drugs.

It is possible to explain the difference found between the 3 × 10 mg Ara C protocol and the single 30-mg injection. In the latter case, we are dealing with a normal bone marrow, in the former, the second and third injections are given to a perturbed population in which CFU-S were triggered into cycle after the first injection [17]. This also explains the more pronounced effect of CFC of the 3 × 10 mg Ara C treatment than of the single injection. In the latter case, cell death is compensated for by entry of CFU-S into this compartment, whereas after the former, the CFU-S compartment is itself depleted. Moreover, some CFC enter the S phase from the G_1 phase between the injections, and more of them are thus killed by the fractionated doses.

On days 4 and 5 after each treatment there was an overshoot of CFU-S and CFC numbers, which was greater after three injections of Ara C than after the single injection. This overshoot observed in the stem cells may be due to cell recruitment and/or to modifications of cell differentiation.

Previous studies [8, 9] have shown that a diffusible factor is secreted after drug treatment, which precedes the entry of quiescent stem cells into cycle. It is possible that some such factors are also secreted that could modify cell differentiation, but we do not thus far have data to corroborate this hypothesis.

In the case of multiple Ara C injections, the overshoot of WBC counts was observed 4 days after that of CFC counts. This interval corresponds quite well with the known transit times between these two compartments.

It is interesting to point out that after the various treatments, the late effects are parallel for CFU-S and CFC. This may be of importance in clinical studies where it is not possible to assess CFU-S. However, in the early hours after treatment the two parameters are not parallel, and it may be premature to conclude that CFC studies alone can suffice.

Another point of interest for clinical studies is the constant relationship between the numbers of CFU-S per 10^5 cells and CFU-S per leg. In patients, the results of CFC are expressed per 10^5 cells, and one must keep in mind that these results are higher than those found per leg.

In spite of the differences found in the various compartments soon after treatment, the late effects are comparable in that there is good restoration 6 months after treatment of all the bone marrow compartments studied. It is yet to be demonstrated that the apparent restoration is real and that the stem cells will react normally to subsequent treatments and not become malignant.

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