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Effect of Some Cytostatics on the Haemopoietic Stem Cells (CFUs) in Blood

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Summary. *The effect of some cell-cycle stage-specific cytostatics on the pluripotent stem cells (CFUs) present in the blood was investigated. The amount of these cells in the blood responds markedly and very quickly to a single administration of any of these drugs. Their effects on the circulating CFUs allowed the drugs tested to be divided into two groups: (1) Hydroxyurea and arabinosyl cytosine induced a profound decrease in the number of CFUs in the blood, which was followed by a return to the normal value and an overshoot that lasted several days; (2) Colchicine, vinblastine, vincristine, and methotrexate first induced an increase in the number of CFUs in the blood, which was followed by a decrease to below the normal values, and still later an overshoot that lasted several days.*

The preliminary data on the effect of hydroxyurea on the CFUs circulating in large quantities in the blood of mice with Friend virus leukaemia indicate that the tumorous stem cells (CFUs) might respond to these cytostatics in a similar way.

Introduction

It was incidentally observed that hydroxyurea, the S phase-specific cytostatic drug, causes a rapid disappearance of the pluripotent haemopoietic stem cells (CFUs) from the blood of mice (Nečas et al., 1978a and b). This effect is surprising since the quiescent population of CFUs is relatively insensitive to killing by the S phase-damaging agents (Becker et al., 1965; Lajtha et al., 1969), which is also true for the CFUs circulating in the blood (Gidáli et al., 1974).

In an attempt to find some explanation for this observation, the effects of several cell-cycle stage-specific cytostatics (hydroxyurea, arabinosyl cytosine, methotrexate, colchicine, vinblastine, and vincristine) on the CFUs present in blood were tested. The results showed that all these drugs influence the number of CFUs in the blood significantly. The drugs tested could be divided into two groups according to the characteristically different response.

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Materials and Methods

Animals

All the experiments were performed with ICR female mice, weighing 20–22 g. The mice were kept in plastic cages in numbers not exceeding 10 mice per cage. The mice were fed pelleted food specially prepared for mice and rats. Food and water were given ad libitum.

Cytostatics

Hydroxyurea. Two preparations were used. The initial experiments were performed with the preparation for clinical use (Litalir, Squibb-Heyden). Later Hydroxyurea, Calbiochem, was used.

Arabinosyl Cytosine. Two preparations for clinical use were used, Cytosar (Upjohn) and Alexan (Mack). Cytosar was dissolved in the accompanying diluent and further diluted with saline, so that the desired dose for a single mouse was contained in 0.5 ml. Alexan was diluted similarly with saline.

Methotrexate. Methotrexate natrium, Lederle, for parenteral application, was used.

Colchicine. Colchicine, Koch-Light Lab., was used.

Vinblastine. Vinblastine, Richter (Hungary), was used. This preparation is produced for clinical use.

Vincristine. Vincristine, Richter (Hungary), was used. This preparation is also for clinical use.

All the drugs used were administered in the saline solution via the IP route. The dilution used was such that a single mouse obtained

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0.5 ml solution. Usually the drugs were dissolved or diluted immediately before the experiment and the solution was stored in a refrigerator at 4° C throughout the experiment. The doses of drugs used are given in the Results section.

Experimental Procedure

Groups of six mice were given a single injection of the cytostatic to be tested at various times before the collection of blood for CFUs determination. The blood was collected from mice anaesthetized with aether by cutting the axillar blood vessels. Heparin was used to prevent blood coagulation. The blood from six mice belonging to the same experimental group was pooled. Shortly after collection, the blood was injected IV to the lethally irradiated (800 R, ^{60}Co source, 28 R/min) recipients (colony-spleen technique: Till and McCulloch, 1961). The number of CFUs in the blood of the given experimental group was measured, nine irradiated mice being used as recipients. Each irradiated mouse was injected with 0.5 ml blood tested. Only in some experiments, in which an increased number of CFUs in the blood could be anticipated, was 0.25 ml blood given. In each experiment a control group of six nontreated mice was included. Blood from mice with Friend virus leukaemia was diluted 100 times before the injection of 0.5 ml per irradiated assay mouse.

Eight days after administration of the blood the irradiated transplanted mice were killed; their spleens were removed and fixed in Bouin's solution, and the numbers of colonies on their surfaces were counted. The mean and standard error of the mean were calculated and compared with those of the control group. On average there were 14 colonies on the spleen of a mouse receiving 0.5 ml of blood from normal mice. The nontransplanted irradiated mice had 0–1 colony per spleen.

The erythrocyte and nucleated cell counts in blood were determined from the pooled blood samples. The sample of blood pooled from six mice undergoing the same experimental treatment was appropriately diluted by Strong's solution for counting erythrocytes or Türk's solution for nucleated cell determination. The cell counts were made in a Bürker chamber.

Results

The Effect of Hydroxyurea

Doses of 100 mg/kg body weight and 1000 mg/kg were tested. At 60 min after adminisistration of the drug there was a decrease in the number of circulating CFUs (Table 1). This fall continued during the ensuing hours, reaching levels lower than 10% of normal in the case of the milder dose used and levels approaching zero in the case of the higher dose (Fig. 1). Afterwards there was a fast return to normal. After the higher dose the effect was investigated until the 8th day. Between days 2 and 8 the number of circulating CFUs exceeded the control level (Fig. 1).

The effect of 1000 mg hydroxyurea/kg on the amount of CFUs circulating in the blood of mice with Friend virus leukaemia is demonstrated in Table 2. At 18 h after hydroxyurea administration the number of CFUs circulating in blood decreased to about 1% of the control value.

Table 1. The effect of hydroxyurea (1000 mg/kg) or colchicine (5 mg/kg) on the CFUs in blood during the first hour following drug administration

Time (min)	Hydroxyurea (%)	Colchicine (%)
15	119 ± 21 n.s.	172 ± 18 $P < 0.02$
30	94 ± 15 n.s.	172 ± 17 $P < 0.02$
60	56 ± 11 $P < 0.05$	256 ± 27 $P < 0.001$

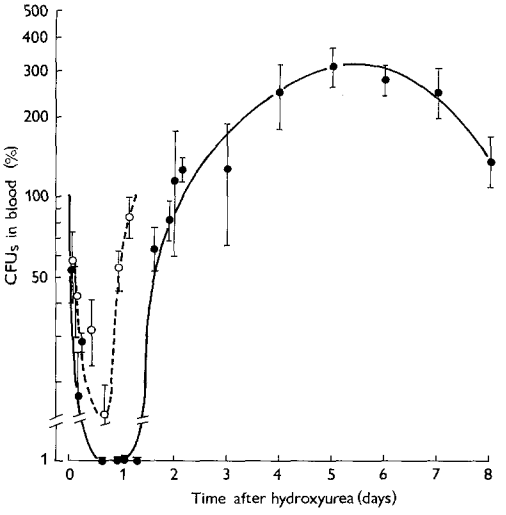

Fig. 1. The time-course of the CFU content in blood after a single injection of hydroxyurea. ●—●, after 1000 mg/kg; ○– – –○, after 100 mg/kg. The *bars* indicate the SEM

Table 2. Effect of hydroxyurea on the colony forming units (CFUs) circulating in blood of mice with Friend leukaemia

	Control	18 h after HU	%
CFUs / ml blood	4,630 ± 530	35 ± 8	1
	5,800 ± 800	72 ± 7	1

Mice of body weight 11 g were injected with the cell-free spleen extract prepared from mice infected with Friend leukaemia virus. These mice were treated with hydroxyurea 2–3 weeks after virus administration

The Effect of Arabinosyl Cytosine

The time course of the CFUs level in the blood after a single injection of arabinosyl cytosine resembled that seen after the administration of hydroxyurea, including the initial fast, pronounced fall (Fig. 2). The doses of 400 mg Cytosar/kg and 300 mg Alexan/kg were used to ascertain that there was no initial rise such as was demonstrated, for example, after methotrexate.

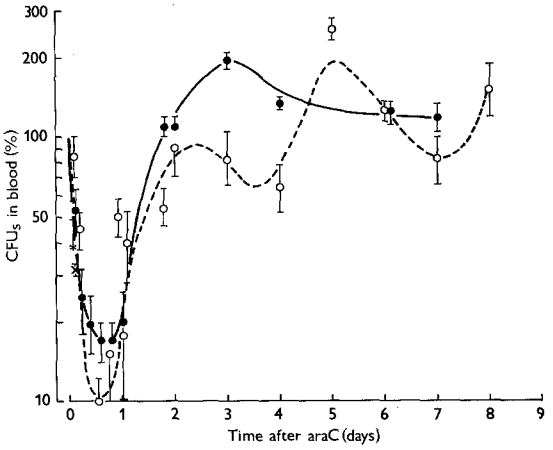

Fig. 2. The time-course of the CFU content in blood after a single injection of arabinosyl cytosine (araC). ●—●, after injection of Alexan in a dose of 100 mg/kg; ×, response to a dose of 300 mg Alexan/kg, measured 3 h after its administration; O- - -O, after injection of Cytosar in a dose of 50 mg/kg; *, response to a dose of 400 mg Cytosar/kg, measured 3 h after its administration. The *bars* indicate the SEM

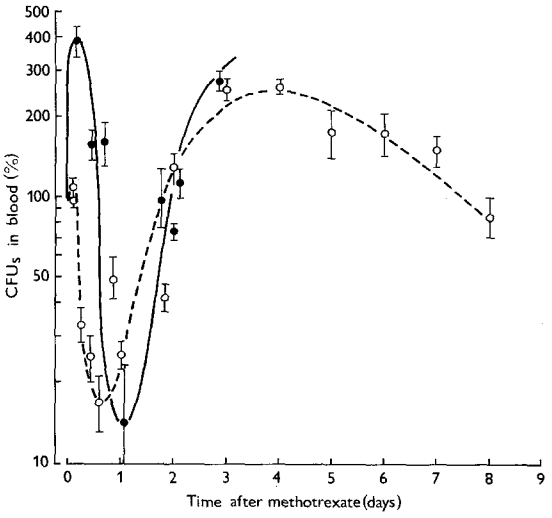

Fig. 3. The time-course of the CFU content in blood after a single injection of methotrexate. ●—●, after 40 mg/kg; O- - -O, after 5 mg/kg. The *bars* indicate the SEM

The Effect of Methotrexate

Two hours after 5 mg methotrexate/kg, the number of circulating CFUs was not significantly different from the control value. There was a steep fall thereafter to approximately 20% of normal. The circulating CFUs returned to normal between days 1 and 2 after the injection. An overshoot was observed between days 2 and 7 (Fig. 3).

After the higher dose (40 mg/kg) there was a significant initial elevation in the number of circulating CFUs

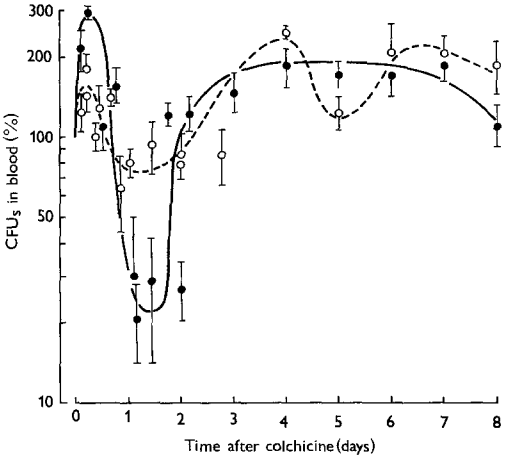

Fig. 4. The time-course of the CFU content in blood after a single injection of colchicine. ●—●, after 2 mg/kg; O- - -O, after 1 mg/kg. The *bars* indicate the SEM

Table 3. CFUs in blood after various doses of colchicine

Dose of colchicine	CFUs/ml blood	
	After 6 h	After 24 h
Control	14	
2 mg/kg	68	4
5 mg/kg	104	34
10 mg/kg	100	66
50 mg/kg	60	Mice died

(Fig. 3). A steep decrease occurred later, circulating CFUs falling to less than 20% of normal 26 h after drug administration. The circulating CFUs returned to normal and an overshoot ensued.

The Effect of Colchicine

Colchicine administration induced an initial increase in the number of circulating CFUs, which could be demonstrated both after 1 mg/kg and after 2 mg/kg. In both cases the increase was followed by a fall to below-normal values (Fig. 4). The overshoot occurred after the third day following drug administration. After the higher dose tested the changes were more pronounced. In an additional experiment it was demonstrated that the number of circulating CFUs increased as early as 15 min after the injection of colchicine (Table 1).

The various doses of colchicine were tested for initial effects. The results are presented in Table 3. The highest

Table 4. Erythrocyte and nucleated cells counts in blood after various doses of colchicine

Dose of colchicine	Erythrocytes (millions/mm³)		Nucleated cells/mm³	
	After 6 h	After 24 h	After 6 h	After 24 h
Control	7.11		3,200	
2 mg/kg	7.33	7.68	5,000	2,300
5 mg/kg	9.52	5.76	4,100	2,500
10 mg/kg	8.03	7.65	8,700	4,800

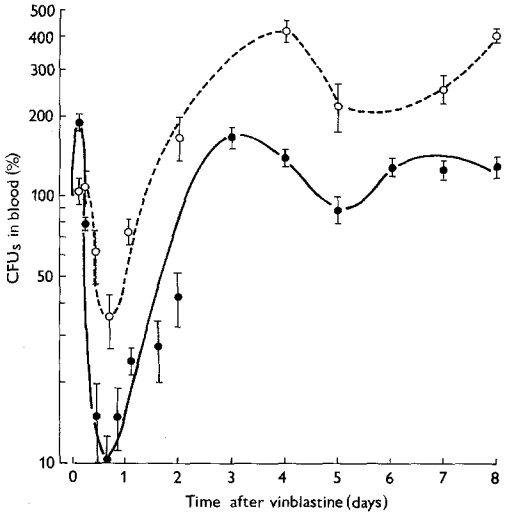

Fig. 5. The time-course of the CFU content in blood after a single injection of vinblastine. ●—●, after 2 mg/kg; O---O after 1 mg/kg. The *bars* indicate the SEM

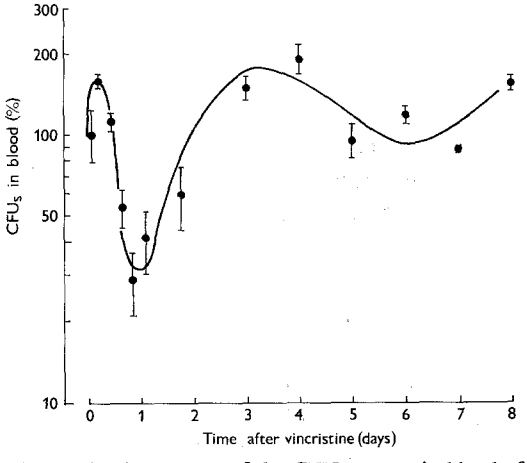

Fig. 6. The time-course of the CFU content in blood after a single injection of vincristine in a dose of 1 mg/kg. The *bars* indicate the SEM

increase in circulating CFUs was observed after the doses of 5 and 10 mg colchicine/kg, when the circulating CFUs increased seven- to eightfold. With these high doses the increase was still evident 24 h after the injection of colchicine (Table 3).

The effect of colchicine administration on blood erythrocyte and nucleated cell counts was also examined (Table 4). No evidence for the significant haemoconcentration was found since the erythrocyte counts did not change. A dose-dependent increase in the nucleated blood cells was observed 6 h after colchicine administration, however.

The Effect of Vinblastine

Vinblastine administration induced an initial increase in the number of circulating CFUs (Fig. 5). After this a fall to below-normal levels was observed. The changes were much more pronounced after the higher dose used (2 mg/kg). Between days 3 and 8 after vinblastine there was a tendency for circulating CFUs to oscillate above normal values.

The Effect of Vincristine

The changes in circulating CFUs, like that occurring after vinblastine, were observed after the administration of 1 mg vincristine/kg (Fig. 6).

Discussion

The presence of CFUs in blood was demonstrated in 1962 (Goodman and Hodgson, 1962). The circulating CFUs probably serve to convey haemopoiesis from the liver into the spleen and bone marrow, which occurs during ontogenesis (Barker, 1970). During adult life the circulating CFUs can be trapped in a suitable cellular environment and in such a place they can give rise to haemopoiesis (Maniatis et al., 1971; Amsel and Dell, 1971). An increased number of circulating CFUs has been observed during recovery from partial-body irradiation (Lord, 1967), following antigenic stimulation (Barnes and Loutit, 1967), after endotoxin administration (Vos et al., 1972), and after some polyanions (Hagenbeek et al., 1976).

Table 5. Average change of the CFUs content of femoral bone marrow after administration of a single injection of the drugs tested in the experiments described

Drug	Dose (mg/kg)	CFUs (% of control)	Period of observation after drug administration (h)
Hydroxyurea[a]	1000	90–50	2–48
Arabinosyl cytosine	50	81	10–28
Methotrexate	5	96	9–40
Colchicine	1	97	5–52
Vinblastine	1	89	8–24

[a] For details see Nečas et al. (1978b)

The cell-cycle stage-specific cytostatics damage the CFUs population in the haemopoietic tissues only slightly, because only a small fraction of these cells is in cycle (Becker et al., 1965; Lajtha et al., 1969; Valeriote and van Putten, 1975; Table 5). In contrast to the bone marrow CFUs, the number of circulating CFUs was significantly affected by a single administration of any of these cell-cycle stage-specific cytostatics (Figs. 1–6). Two different responses were observed:

(1) Hydroxyurea and arabinosyl cytosine induced a rapid and profound decrease in the amount of circulating CFUs. The nadir of this decrease occurred between 12 and 24 h after the drug administration.

(2) Methotrexate, colchicine, vinblastine, and vincristine first induced an increase in the number of circulating CFUs, an effect that was not observed after hydroxyurea or arabinosyl cytosine.

The precise mechanism by which the drugs tested affect the circulating CFUs is not clear at present. The changes demonstrated probably reflect the imbalance between the release of CFUs into the blood from the haemopoietic tissues and the seeding of these cells in the tissues.

Since the absolute number of CFUs circulating in the blood of normal mice is low (20–40 CFUs/ml blood), a single injection of hydroxyurea was given to mice previously infected with Friend virus (Friend, 1957). There were about 5000 colony-forming units in 1 ml blood from these mice 2–3 weeks after infection. Following hydroxyurea administration the number of these cells in the blood decreased to very low levels, like CFUs in the blood of normal mice (Table 2).

The present results allow the following conclusions:

(1) The number of circulating CFUs (normal and leukaemic) responds sensitively to the administration of cell-cycle-specific cytostatics.

(2) The response observed during the first few hours differs characteristically for various cell-cycle stage-specific cytostatics.

(3) The response of circulating CFUs to the cell-cycle-specific cytostatics differs markedly from that of bone marrow CFUs.

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