Mode of Action of Chemotherapy In Vivo on Human Acute Leukemia

I. DAUNOMYCIN

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ABSTRACT The leukocytes of 16 adult patients with acute myeloblastic leukemia were studied by autoradiographic methods to elucidate the mode of action of daunomycin.

It was shown that daunomycin, at clinically useful doses, exhibits a cytolytic effect on all leukemic blasts whatever their cell-cycle phase. This cytolytic action affects, however, preferentially S-phase cells.

It was shown also that blasts of patients less sensitive to daunomycin or receiving a lesser dose of the drug are temporarily blocked in G_2 phase (delayed mitosis) or in G_1 phase (prolonged generation time).

Finally daunomycin appeared to hamper the passage of G_2-blocked blasts from the bone marrow to the blood, while G_1-phase cells crossed freely.

INTRODUCTION

Daunomycin is one of the first drugs capable of inducing remissions in human acute myeloblastic leukemia with a high frequency (1). It has been shown to possess antimitotic activity on mammalian cells (2) and an inhibitory effect on RNA and DNA synthesis in bacteria (3), in various experimental tumors (4), and in human lymphocytes (5). The maximal lethal effect occurs in HeLa cell cultures in S phase and is less marked during the G_1 phase (6).

However there are no published studies on the mechanism of action of daunomycin on the leukocytes of human acute myeloblastic leukemia. Therefore, the main purpose of this work was to carry out such a study and to determine whether, under clinical conditions, this drug is capable of killing out-of-cycle leukemic blasts. Standard doses of the drug were used and classical parameters of cell proliferation were investigated.

METHODS

16 leukemic patients were studied. Except for patients 4 and 16 (acute myelomonocytic), and patient 10 (acute promyelocytic), all were of the acute myeloblastic variety. All the patients were studied at the time of diagnosis and had never been treated with antileukemic drugs before.

Patients one to nine received intravenously (i.v.) tritiated thymidine ([^{3}H]TdR) (Radiochemical Center, Amersham, England; Sp act 5 Ci/mmol) (1 mCi/10 kg body weight) at time 0, and daunomycin (60 to 80 mg/m^2 body surface area [BSA])[1] either 1 h later (patients one to seven) or 24 h later (patients eight and nine). Initial flash-labeling indexes were determined on blood and bone marrow (BM) samples taken 30–60 min after [^{3}H]TdR.

Patients 10–13 received as treatment 70–80 mg/m^2 BSA daunomycin at time 0. Serial samples of blood and BM were taken before and repeatedly after the treatment and incubated for 1 h with [^{3}H]TdR (10 μCi/ml) to determine in vitro labeling indexes.

In patients 14 and 15, the "natural" turnover of blood blasts before the administration of any drug was first studied as follows: ½ liter of blood was collected in an ACD-A-containing plastic bag (Fenwal Lab. Division of Travenol Lab., Inc., Morton Grove, Ill.), incubated for 1 h at 37°C with tritiated cytidine ([^{3}H]Cyt) (Radiochemical Center, Amersham, England, Sp act 27 Ci/mmol) (0.5 mCi) and rapidly autotransfused. Blood samples were drawn from

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[1] *Abbreviations used in this paper:* BM, bone marrow; BSA, body surface area; I_L, labeling index; I_M, mitotic index; MGC, mean grain count.

the bag and serially from the patient during the following days, for the preparation of blood smears. 4–5 days later daunomycin was injected and leukocytes and differential counts were performed repeatedly.

A technique similar to that used in patients 14 and 15 was used in patient 16 except that the in vitro incubation lasted 2 h and that daunomycin was injected immediately after blood withdrawal, i.e., 2 h before autotransfusion.

Blood smears were done from leukocytes buffy coat, BM smears were obtained from marrow spicules. The smears were fixed in methanol for 30 min, dipped in a photographic emulsion (Kodak NTB2, Eastman Kodak Co., Rochester, N. Y.), and exposed in the dark for several weeks at 4°C. Developing and staining were carried out by the classical method.

The labeling index (I_L) was determined by observing from 1000 to 5000 blasts per smear depending on the blast concentration on the smear. In the marrow, as in the blood, a cell was considered as labeled when more than three grains were seen over the nucleus. This definition was used in all cases except in patients two and nine for whom a threshold of five and four grains, respectively, was used because the background happened to be higher in the two cases. The mean grain count (MGC) of the labeled cells was determined in the BM of patients three through six, eight and nine.

In patient nine the MGC was determined for 10 days, from day 2, i.e. after the first division, until day 11. Since the daughter cells of lightly labeled cells may fall below the counting threshold upon cell division, their exclusion by the usual method could artificially raise the MGC. To correct this, the fraction of labeled cells used for the MGC estimate was increased proportionally to the decrease in I_L (10).

The mitotic index (I_M) was determined by counting the number of blasts in mitosis among 5000 blasts on direct smears. In patient three, only 2500 and 2000 blasts, respectively, could be observed 4 and 7 h after daunomycin administration. The mitotic labeling indexes were determined from a small number of mitoses.

RESULTS

Table I summarizes the main laboratory data on the 16 patients studied.

Study of the effect of daunomycin on the fate of blasts prelabeled by [³H]TdR in vivo (Tables II and III and Figs. 1 to 4). Table II lists the I_L in the blood of patients one to nine after an injection of [³H]TdR. Daunomycin was given either 1 h (patients one to seven) or 24 h (patients eight and nine) after [³H]-TdR. The results of Table II, normalized, are represented in Fig. 1. Patients eight and nine, while untreated, showed an increase in the percentage of labeled blasts in the blood and daunomycin given 24 h after [³H]TdR did not interrupt this release of blasts from the BM to the blood. On the other hand, patients one to seven, receiving daunomycin 1 h after [³H]TdR, showed a partial or complete interruption of the release of labeled blasts to the blood for a period of 48 h. Therefore daunomycin prevents the passage of blast from BM to the blood only if it is given before mitosis. It has no action on the passage of [³H]TdR-labeled cells

TABLE I
Main Laboratory Data of Patients

Patient		Type of leukemia*	Leukocytes/ mm³	Blasts in blood	Blasts in marrow	Labeled blasts in blood	Labeled blasts in marrow	ARG exposure	Daunomycin
				%	%	%	%	*days*	*mg/m²*
[³H]TdR in vivo									
Ba	1	AML	119,000	54	66	5.9	—	18	80
Bro	2	AML	220,000	93	88	2.4	—	57	70
Cat	3	AML	13,300	77.5	63.5	1.4	10.4	21	70
Hans	4	AMML	48,000	87	49.5	1.7	11.4	44	80
Sney	5	AML	240,000	48	35	7.2	8.9	17	70
VL	6	AML	7,100	80	50.5	0.8	8.4	32	70
VD	7	AML	21,400	79	80	1.8	7	103	60
Maq	8	AML	4,700	53	60	1.2	9.8	36	80
Atti	9	AML	23,300	53.5	73	0.3	7.7	18	80
[³H]TdR in vitro									
Cal	10	APML	19,000			4.8	36	18	70
VAch	11	AML	500				11.9	18	80
Ro	12	AML	1,900				4.6	18	80
Poi	13	AML	3,600				10.4	18	80
[³H]Cyt in vitro-vivo									
VR	14	AML	76,000	96				29	80
Ca	15	AML	90,000	91				32	70
Gen	16	AMML	105,800	92				43	80

* AML, acute myeloblastic leukemia. AMML, acute myelo-monoblastic leukemia. APML, acute promyelocytic leukemia.

Table II

Percentage of Labeled Blasts in the Blood after an i.v. Injection of [³H]TdR at Time (t) Zero

Daunomycin injected 1 h after [³H]TdR														Idem 24 h after [³H]TdR			
Patient 1		Patient 2		Patient 3		Patient 4		Patient 5		Patient 6		Patient 7		Patient 8		Patient 9	
t	%	*t*	%	*t*	%	*t*	%	*t*	%	*t*	%	*t*	%	*t*	%	*t*	%
1h	5.9	1h	2.4	1h	1.4	1h	1.7	1h	7.2	1h	0.8	0h35	1.8	1h	1.2	1h	0.3
5h20	6.5	7h10	3.4	3h	1.1	3h30	1.2	3h20	5	3h	0.2	4h20	2.2	2h05	2.9	2h10	0.2
7h35	3.2	11h25	3	5h	1.3	7h30	1.3	8h	3.6	8h	0.6	23h	2	3h50	1.2	3h55	0
24h	0.8	24h	2.6	7h	1.5	9h20	1.6	12h	4.7	11h40	0.6	47h	6	6h05	2.6	6h10	0.1
29h20	1.7	48h50	3	9h	0.9	16h	2.6	25h	4.1	19h30	0.8	78h	11.2	9h	4.3	9h05	1.4
54h	2.6	71h	4	13h45	1.4	19h	4	32h	5.6			95h	9.8	11h45	5.8	12h05	1.2
		95h	1.6	23h	0.4	48h	6.8	49h	11			119h	8.2	14h20	6.6	14h20	1.5
		100h	2.8	31h	1	54h	5.3	68h	3.6			143h	7.6	21h35	9.4	22h50	2.5
		119h	2	48h	1.4	64h	5.4	115h	4.8			173h	3	23h20	8.8	24h35	3.6
				73h	1.2	73h	6	139h	2.2			193h	1.8	33h30	12.8	27h05	3.1
				293h	0.2	87h	5.5	188h	0.8			269h	0	48h	14.2	34h30	4.2
								236h	1.6					61h	14.1	49h	8.3
								308h	0.4					72h	16.2	60h	7.4
																73h	8.7
																77h	8.9

which were given a sufficient time to complete DNA synthesis and mitosis, i.e. G_1 cells.

In the BM there was also evidence that blasts, at least in some patients, were more affected in one phase of the cell cycle than in the others. This is seen on Table III and Fig. 2. Table III shows the I_L and the MGC

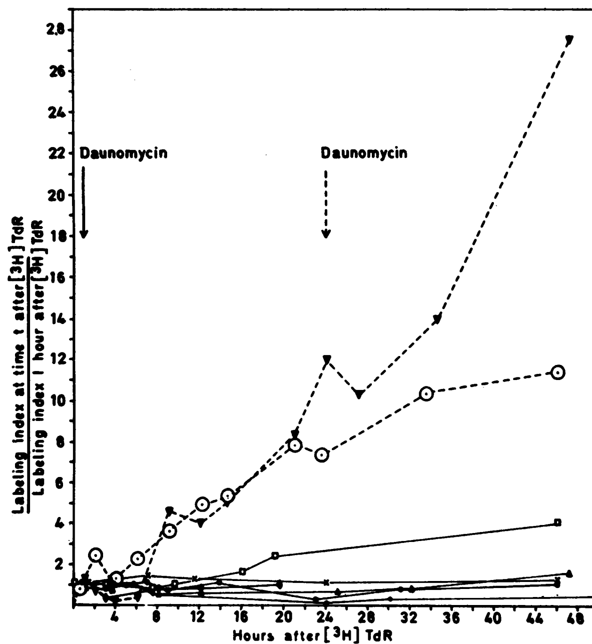

Figure 1 Evolution of the [³H]TdR I_L (normalized) of circulating blasts labeled in vivo in patients one (●), two (×), four (□), five (△), and six (◆) who received daunomycin (→) 1 h after labeling and in patients eight (⊙) and nine (▼) who received daunomycin (-- →) 24 h after labeling.

in the BM of patients three to six who received daunomycin 1 h after [³H]TdR and of patients eight and nine who received daunomycin 24 h after [³H]TdR. The I_L, normalized, is illustrated on Fig. 2. In patients eight and nine, untreated for 24 h, the I_L of the blasts increased in the marrow, while in patients three to six, treated 1 h after [³H]TdR, no such increase of the I_L was observed. In patient three, a 95% decrease of the I_L from its original value was observed 72 h after daunomycin.

If on the one hand there was evidence for a cytolytic effect, then on the other hand, some mitotic activity persisted and progressively recovered, (Table III and Figs. 3 and 4) indicating that the drug had also a cytostatic effect. Fig. 3 illustrates the rapid fall of the number of mitoses after the injection of daunomycin in the BM of patients three and four. A few labeled mitoses were still observed in patient four at 73 h, but this was not true for patient three. Fig. 4 shows the evolution of the MGC of the blasts seen in the blood and marrow of patient nine, who received daunomycin 24 h after [³H]TdR. For 10 days a stable MGC both in marrow and blood blasts was observed indicating a complete absence of division during this period of time, as a consequence of daunomycin.

Study, by means of in vitro ³HTdR labeling, of the effect of daunomycin on the capacity of leukemic blasts to enter proliferative activity (Table IV and Fig. 5). This experiment has shown that the effect of daunomycin was to decrease the capacity of the leukemic blasts to enter DNA synthesis in three patients out of four and to increase it in one. Table IV shows the in vitro I_L of marrow blasts at several moments after daunomycin injection (patients 10–13). Fig. 5 is a

graphical representation of the results of Table IV, normalized. In patients 10, 12, and 13 the marrow I_L of blasts decreased significantly, while it increased in patient 11. In this last patient an I_M could be determined at time 0 (0.08%) and at 120 h (0.46%).

Study of the effect of daunomycin on nonproliferating blasts, using in vitro labeling of blood by [^{3}H]Cyt (Figs. 6-8). Fig. 6 depicts on semilogarithmic paper the decrease of the number of circulating blasts, after an injection of daunomycin. Patient six only presented a sustained increase of her blast count for 20 h before death. In the other patients, exponential decreases were observed with $t_{1/2}$ ranging from 6 to 31 h.

In patients 14 and 15, the $t_{1/2}$ was also determined before daunomycin by a method which could not be used again after the administration of daunomycin, since this drug is known to affect RNA metabolism (4, 5) and thereby perhaps the labeling procedure. In these patients, the $t_{1/2}$ of blood blasts before daunomycin treatment, as determined from the decrease of the number of autotransfused in vitro-labeled blood blasts, was then compared to the $t_{1/2}$ after daunomycin treatment, as determined from the decrease of the number of circulating blasts. In patient 14 it was 40 h before and 31 h during treatment. In patient 15 the $t_{1/2}$ were even more different namely 74 h and 14 h as shown on Fig. 7. The $t_{1/2}$ was thus shorter after daunomycin in both cases indicating that this drug accelerates the "loss" of cells from the blood.

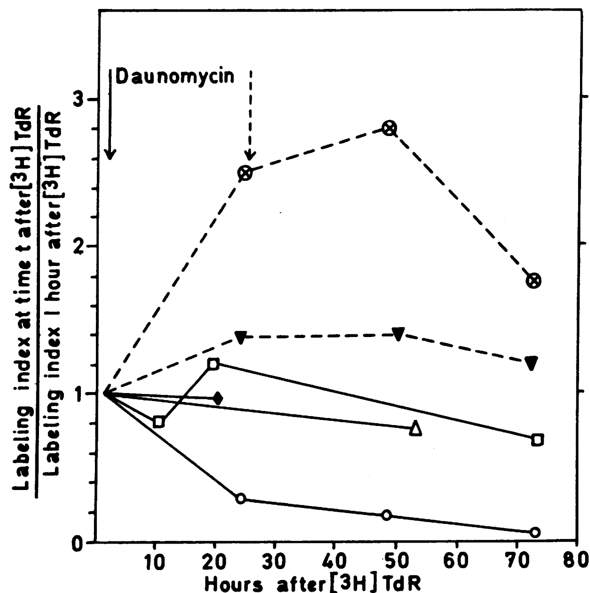

FIGURE 2 Evolution of BM I_L (normalized) of blasts after [^{3}H]TdR i.v. Patients three (○), four (□), five (△), and six (◆) received daunomycin (→) 1 h after [^{3}H]TdR i.v., while patients eight (⊗) and nine (▼) received daunomycin (- - →) 24 h after [^{3}H]TdR i.v.

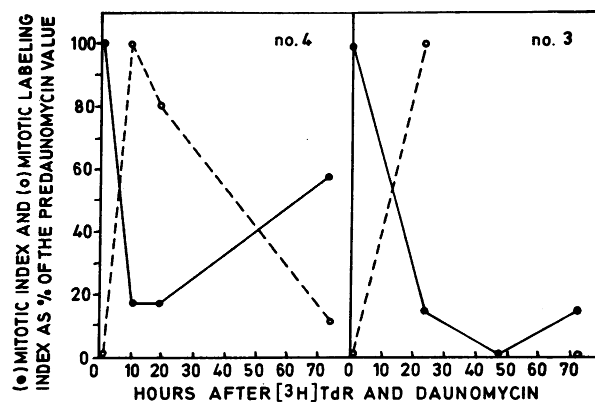

FIGURE 3 Evolution of the I_M and mitotic I_L of marrow leukemic blasts after an injection of daunomycin to patients four and three.

TABLE III

Evolution of I_L and MGC BM Blasts. Patients three to six Received Daunomycin 1 h after Labeling, while Patients eight and nine Received Daunomycin after 24 h

H after [^{3}H]TdR	Blast I_L	MGC
Patient 3		
1h	10.4	16.4
24h	2.9	14.4
48h	1.9	17.1
72h40	0.4	
Patient 4		
1h	11.4	19.3
10h	9.1	17.1
19h	13.6	16.8
73h	7.7	13.3
Patient 5		
1h	8.9	21.4
49h	6.9	10
Patient 6		
1h	8.4	24.6
19h30	7.5	13.9
Patient 8		
1h	9.8	20.3
23h30	24.4	14.3
48h	27.4	18.7
72h	17.2	10.9
Patient 9		
1h	7.7	
24h30	10.6	15
49h	10.5	13.2
73h		12

TABLE IV

Percentage of In Vitro-Labeled Blasts in the Marrow before (t = 0) and at Different Times After an Injection of Daunomycin

Time after daunomycin injection	Patient			
	10	11	12	13
h				
0	36	11.9	10.4	4.6
6	21			
9	13		7.4	
24		12.9	5.6	
29				1.1
48		16.6		
78				2.8
96			4.9	
120		22.6		

In order to investigate whether this loss of blasts from the blood was a direct or indirect effect of the drug, the following experiment was performed in patient 16: approximately 10% of the circulating blast cells were incubated with [^{3}H]Cyt outside the body while daunomycin was injected into the patient. 2 h after this injection the labeled cells were autotransfused. Fig. 8 shows that the percentage of these labeled blasts in the blood increased progressively indicating that the labeled cells were decreasing more slowly than the unlabeled cells, i.e. those exposed to the full dose of daunomycin and thereby that the decrease observed must represent a direct effect of the drug.

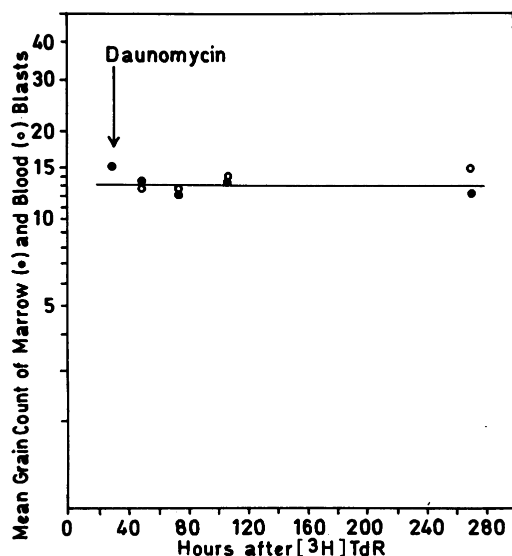

FIGURE 4 MGC in BM (●) and blood (○) blasts of patient nine receiving daunomycin 24 h after [^{3}H]TdR i.v.

DISCUSSION

In human acute myeloblastic leukemia, active drugs manifest their effect by a decrease in the number of blasts present in the body. This can only be achieved through an effect on the cells themselves and/or on their proliferation mechanism. Antileukemic drugs can therefore be variously classified by their killing or blocking effects on cells according to concepts originating in antimicrobial research, or by their differential effect at different phases of the cell cycle. For human therapeutic purposes, only clinically useful doses and schemes of drug administration are of interest and observations on humans necessarily limits the spectrum and the number of feasible studies. Fortunately the use of autoradiographic methods of observation of blood cells appears very suitable for the study of the mechanism of action of antileukemic drugs because clear lines can be drawn between different phases of the cell cycle and between different types of action on cells. Our data will therefore be discussed under the following headings.

Evidence for cytolytic and temporary cytostatic effect on S or G_2 cells (Table III and Figs. 2 and 3). That daunomycin has a marked cytolytic effect on blasts in S or G_2 phase is most evident in the study of patient three (Table III and Fig. 2). Indeed, the percentage of labeled cells in the marrow at 72 h (patient three) was seen to have dropped to approximately 5% of its original value. This cannot be due to a dilution of these

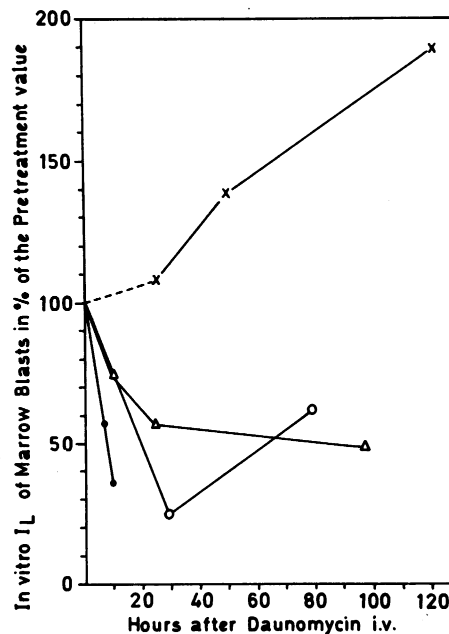

FIGURE 5 In vitro [^{3}H]TdR I_L of marrow blasts of patients 10 (●), 11 (×), 12 (○), and 13 (□) treated by daunomycin at time 0.

cells by the division of nonlabeled cells since the I_M decreased considerably at the same time (Fig. 3). The observation that the few mitoses seen again at 72 h were all unlabeled supports the idea that by then the labeled cells were unable to divide, and were probably dead.

On the other hand, patient four did not show a marked drop of the I_L of the blasts in the marrow as patient three. Moreover, the successive MGC of the labeled blasts in the BM of patient four (Table III) show that these cells were dividing, although slowly since one halving of the MGC was not yet observed at 73 h. At that time and in the same patient, when the I_M started to increase, a few mitoses were still labeled (Fig. 3, patient four), and this confirms that the decreasing MGC was due to cell division in accordance with the hypothesis that these cells had been only temporarily blocked in S or G_2 phase. The progressive release of this block also explains a slow and delayed raise of the blood I_L of patient four (Fig. 1). Patient seven, who received a smaller dose (60 mg/m²) than the other patients, showed also a delayed release of labeled cells from the marrow into the blood after 24 h (Table IV) as opposed the other patients who did not show significant release during the 48 h of observation.

Thus, besides a lytic effect in S phase (patient three), daunomycin also shows a blocking effect on blasts (patients four and seven). We suggest that the blocking and killing effects of daunomycin probably represent stages in a spectrum ranging from an undisturbed cell to a destroyed cell, depending, on the one hand, on the dose of daunomycin and on the other hand, on the cell sensitivity (of which cell-cycle phase sensitivity is one aspect).

The method used cannot differentiate between a S or a G_2 phase block. Several in vitro studies have indi-

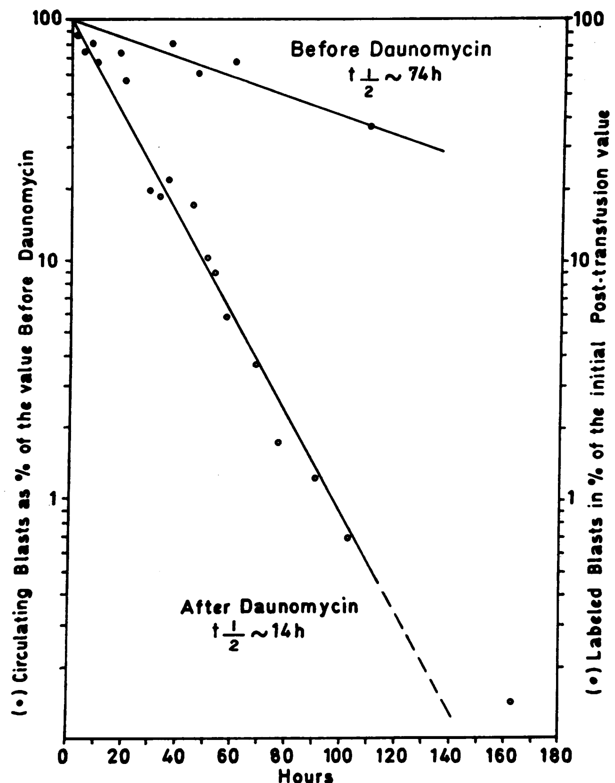

FIGURE 7 $t_{\frac{1}{2}}$ of blood blasts of patient 15 before and after daunomycin.

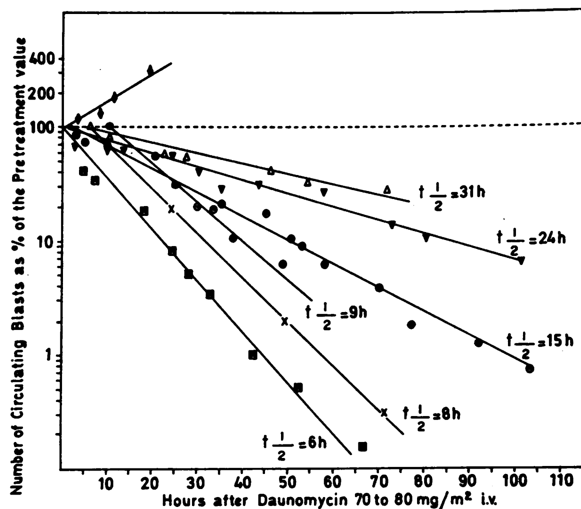

FIGURE 6 Effect of daunomycin on the number of circulating blasts. Patients 2 (×), 6 (♦), 8 (⊗), 9 (▼), 14 (△), 15 (⊙), and 16 (⊠).

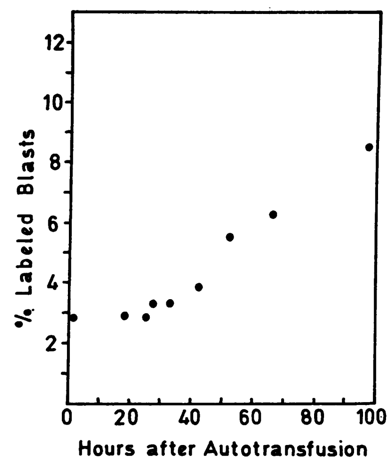

FIGURE 8 Patient 16: a fraction of the blood blast was labeled with [³H]Cyt outside the body while daunomycin was injected to the patient. The labeled blasts were autotransfused 2 h later. I_L were then counted in the blood.

cated G_2 phase as the most probable site (5, 7). It has been observed in vitro that small doses of daunomycin may cause a rapid drop in the I_M without modifying the [^{3}H]TdR uptake, which is affected at higher doses only (7). This would be in favor of a G_2-block, possibly by inhibition of RNA synthesis during this phase (8).

Evidence for a cytolytic effect on G_1 phase cells in the blood (Figs. 6–8). Serial blood counting after administration of daunomycin in most patients (Fig. 6) showed a rapid exponential fall of the number of circulating blasts which are essentially G_1-phase cells. The studies on patients 14 and 15 demonstrate that this decrease was due to an accelerated loss of cells from the blood; patient 15 is shown in Fig. 7 as an example. In both patients the $t_{\frac{1}{2}}$ of this decrease was shorter than the "natural" $t_{\frac{1}{2}}$ of blasts determined in the blood of the same patient before treatment. If, for instance, daunomycin were only halting the production of new blasts in the marrow and thereby the influx of blasts in the blood, both $t_{\frac{1}{2}}$ would be identical. Moreover, the observation that daunomycin did not inhibit the release into the blood of marrow blasts in G_1 phase, as shown in Fig. 1 (patients eight and nine), should give an even longer $t_{\frac{1}{2}}$ after daunomycin than before daunomycin administration if the processes of egress of blasts from the blood were undisturbed. In fact, the $t_{\frac{1}{2}}$ was shorter after daunomycin and this indicates that the flux of cells out of the blood was accelerated by the treatment.

Two alternative explanations may be proposed for this observation: firstly, a lytic effect on blood blasts, i.e. essentially G_1-phase blasts, or secondly, a redistribution of blood blasts to extravascular sites (including the BM sites) preliminarily depleted of cells as a consequence of the treatment. The second alternative, namely a redistribution of blood blasts due to treatment, has been observed in one patient treated by methotrexate (9). The explanation proposed for this was that the treatment reduces the total blast population in the body and thereby, through a population-size sensing mechanism, indirectly stimulates the proliferation of the surviving cells and their migration to sites favorable for this activity. To test this mechanism as an explanation for our data, in vitro-labeled blasts, unexposed to daunomycin, were autotransfused to patient 16 who had received 2 h earlier a high dose of daunomycin. The I_L in the blood after autotransfusion (Fig. 8) increased significantly for 4 days while the number of blood blasts decreased with a $t_{\frac{1}{2}}$ of 6 h (Fig. 6). This indicates that the blasts exposed to the drug (unlabeled) and those not exposed (labeled) behaved differently, and therefore invalidates the hypothesis of a redistribution of cells through an indirect mechanism, which would affect identically exposed and nonexposed cells. Moreover, 95 h after autotransfusion of labeled cells, the BM showed only 0.2% labeled cells instead of 8% in the blood. No evidence for a return of circulating blasts to the marrow after daunomycin could be demonstrated in this way.

In view of the data presented in the above paragraph, a lytic effect on the cells in G_1 phase seems to be the only possible explanation for the observed decrease of the total number of blood blasts after daunomycin.

The observation that in the blood the number of circulating blasts after daunomycin decreased exponentially over more than 2 log 10 (Fig. 7) indicates that at least 99% of these cells were affected identically by the drug, which means that practically all the blasts irrespective of their position in the cell-cycle were affected.

Evidence for either a cytostatic or activating effect of daunomycin on G_1-phase cells (Table IV and Figs. 4 and 5). Patient nine, who received daunomycin 24 h after [^{3}H]TdR, i.e. when labeled cells had reached G_1 phase, showed a stable MGC of his blasts in the blood and in the marrow for 240 h (Fig. 4). This duration contrasts with the results obtained by Clarkson, Ohkita, Ota, and Fried (10) in 16 untreated leukemic patients. In these patients the MGC decreased by 50% in 76 to 215 h. The present study indicates that in our patient no division of blast cells took place for 10 days but does not indicate in which phase the cells were blocked. The study of patients 10, 12, and 13 with a decreased in vitro [^{3}H]TdR flash-labeling index in their BM up to 96 h after daunomycin administration (Table IV and Fig. 5) permits then to conclude that leukemic blasts can be blocked in G_1 phase by this drug.

The paradoxical increase of the flash labeling of the blasts in the marrow of patient 11, also shown on Fig. 5, presumably represents an acceleration of proliferation since the I_M of these cells also increased. This effect may be considered as similar to the increased mitotic activity observed after extracorporeal irradiation of the blood (11), methotrexate (12), or cytosine arabinose therapy (13). It is generally considered as a response, through a maintained regulation mechanism, to a sudden reduction of the blasts population consecutive to the treatment.

Effect of daunomycin on the passage of blasts from the marrow to the blood (Tables I and II and Fig. 1). Our results, shown in Table I, confirm several studies which have shown a considerably higher I_L of blasts in the BM than in the peripheral blood. This gradient allows the study of the release of blasts from the marrow into the blood after their in vivo labeling by [^{3}H] TdR (14). Fig. 1 illustrates the progressive replacement of unlabeled blood blasts by labeled blasts originating from the marrow in patients eight and nine untreated over 24 h. The injection of daunomycin 1 h after

[^{3}H]TdR (patients one to seven) while labeled blasts were either still in S or a few already in G_2 phase, prevented the rise of the I_L in the blood.

On the other hand, labeled blasts keep on appearing in the blood, simply by delaying the injection of daunomycin for 24 h after [^{3}H]TdR. Actually, this delay allows the labeled cells, to complete their DNA synthesis which takes about 20 h (10) and to access to the G_1 phase after the passage through G_2 and M phases which in untreated patients last approximately 2 to 4 h.

In the case of patient three, the absence of release of labeled blasts from the marrow into the blood is easily explained by the destruction and elimination of these cells from the marrow. In the case of patient four, labeled cells were still present in the BM after the administration of daunomycin; nevertheless these labeled cells were not released into the blood. A proposed explanation of this fact is that the release from the marrow may be cell-cycle phase dependent: cells in S or G_2 phase would stay in the marrow and be released only when in G_1 phase, after cell division. The blocking action of daunomycin in S or G_2 phase would therefore impair the release. This may represent a cell-size-dependent phenomenon as cell division reduces the cell volume by a factor of 2. This hypothesis is in agreement with observations made on patient eight that blast cells were smaller in the blood than in the BM. (Unpublished observation). As a matter of fact, in this patient, before any treatment, the surfaces of the blood blasts varied between 160 and 600 arbitrary U with a median of 360, whereas the marrow blasts surface ranged between 200 and 960 with a median of 430.

Our mechanistic explanation for the release of marrow blasts into the blood is further supported by electron microscope studies which have shown that passage proceeds through ostia with a relatively low aperture (15) and by elastometric studies which have shown that myeloblasts are much less deformable than more mature granulocytes (16). It would also provide an understanding for the low I_L always observed in blood as opposed to BM. Many authors have shown that the proliferative activity of small blasts is low. Therefore, if small blasts are preferentially released from the marrow, the low I_L in the blood would not be surprising.

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