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Adriamycin Distribution in Plasma and Blood Cells of Cancer Patients with Altered Hematocrit*

E. PIAZZA,† M. BROGGINI,‡ A. TRABATTONI,§ N. NATALE,§ A. LIBRETTI† and M. G. DONELLI‡‖

†*V[a] Cattedra di Patologia Speciale Medica, Ospedale L. Sacco, Università di Milano, Milan, Italy*

‡*Istituto di Ricerche Farmacologiche "Mario Negri" Milan, Italy*

§*Divisione di Ostetricia e Ginecologia, Ospedale L. Sacco, Università di Milano, Milan, Italy*

Abstract—*Adriamycin (AM) distribution in the cellular and plasma components of blood has been investigated in cancer patients with hematocrit values ranging from 23 to 49 after i.v. drug doses (40–70 mg/m^2). AM was measured by a fluorimetric technique in total blood, plasma and blood cells. Blood levels were found to be related to the dose, and when the number of blood cells per milliliter was low, a smaller amount of AM was found in the cell fraction and a larger amount was found in the plasma fraction. A further result of these studies was that, as already shown in Walker-bearing rats, AM accumulated to a marked extent in blood cells, particularly platelets, as expressed by the drug concentration per unit volume, and persisted longer in white blood cells and platelets than red cells, suggesting that the various cells have different roles in transport, storage or metabolism of the compound.*

INTRODUCTION

IT HAS been reported in *in vivo* and *in vitro* studies that blood cells may have a role as carrier [1–6], metabolic [7, 8] or storage sites of drugs [4, 6, 9–11]. A preceding paper by this laboratory [12] described the distribution of adriamycin (AM) in the blood components of rats [plasma, red cells (RBC), white cells (WBC) and platelets (PT)], indicating that the drug concentration in the various blood cell types accounts for more than 50% of the total drug concentration in blood and that these drugs accumulate in RBC, WBC and particularly PT, against a gradient. In humans, too, sustained AM levels, even higher than in plasma, are observable [13] in blood cells, leukocytes seemingly concentrating the drug to a much greater extent than erythrocytes [14].

It was also reported in a later paper by our group [1] that hematological pathology, in particular lower hematocrit (HT) values usually associated with the presence of a tumor, greatly alters the relative distribution of AM to plasma and blood cells of Walker-bearing rats, as much more of the compound becomes available in the plasma when the cellular component accounts for a smaller volume.

Since the role of blood cells in AM transport and storage is not yet defined, the unbound form of the compound present in plasma is the main candidate for drug activity. It is therefore reasonable to assume that changes in drug distribution between plasma and the cellular fraction may have a bearing on therapeutic or toxic effects. In view of the wide clinical use of AM in the therapy of a series of neoplastic diseases, these considerations prompted us to investigate the relative distribution of AM to plasma and blood cells of patients suffering from cancer and showing low HT values.

MATERIALS AND METHODS

Patients and treatment

Fourteen patients, 9 females and 5 males aged between 23–71 yr, with solid tumors (lung, breast, uterine or ovarian cancer), receiving AM treatment as single or multidrug therapy, were admitted to this study (see Table 1). The

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‖All correspondence to: Dr. M. G. Donelli, Istituto di Ricerche Farmacologiche, "Mario Negri", Via Eritrea, 62, 20157 Milan, Italy.

Table 1. History of cancer patients submitted to AM treatment

No.	Pat.	Sex	Age	Diagnosis	Previous therapy	AM mg/m² i.v.	Cycle	Concomitant therapy mg/m² i.v.	Hematological parameters Ht %	RBC ×10³/mm³	Hb mg/100 ml	WBC ×10³/mm³	PT ×10³/mm³
1	G.C.	F	61	Ovarian CA	PR,CTX	40	3	—	28	2.800	8	2.2	200
2	S.D.	F	23	Ovarian CA	TBHSO,TCT,CTX	40	3	—	36	3.700	11.8	3.0	180
3	B.A.	M	62	Lung CA	—	40	3	CTX600 day 1 + BCNU 50 q 4 wks	49	5.200	16.2	9.0	230
4	R.A.	F	70	Breast CA	—	40	3	CTX600 day 1 + 5FU500 day 1, 7, 14 q 4 wks	45	5.000	14.8	6.8	320
5	B.G.	M	69	Lung CA	Lobectomy	70	1	—	42	4.200	14.0	6.6	170
6	P.M.	F	58	Uterine leiomyosarcoma	TBHSO	60	1	DTIC 150 day 1 q 3 wks	31	4.000	10.4	3.6	260
7	M.F.	F	67	Ovarian CA	TBHSO	70	4	—	37	4.400	12.2	6.4	200
8	G.F.	F	67	Ovarian CA	TBHSO	70	5	—	39	4.800	13	7.4	300
9	C.E.	F	55	Uterine leiomyosarcoma	TBHSO	60	1	DTIC 150 day 1 q 3 wks	38	4.300	12.8	6.6	240
10	B.I.	F	66	Ovarian CA	TBHSO	70	4	—	33	3.400	10.8	4.4	280
11	P.L.	M	65	Lung CA	Lobectomy	70	1	—	40	4.800	13.2	17.6	270
12	M.M.	F	71	Ovarian CA	PR	40	1	—	23	3.200	10	16.0	220
13	A.C.	M	54	Lung CA	Pleurectomy	70	1	—	36	4.200	12	10.0	280
14	F.V.	M	67	Lung CA	BLM + MTX + VCR 6 cycles	70	1	—	38	4.600	12.8	6.4	260

PR = partial removal; CTX = cyclophosphamide; TBHSO = total bilateral hysterectomy salpingoophorectomy; TCT = telecobalt therapy; BCNU = 1,3-bis-(2-chloro-ethyl)-1-nitrosourea; 5FU = 5-fluorouracil; DTIC = 5(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide; BLM = bleomycin; MTX = methotrexate; VCR = vincristine; Ht = hematocrit (n.v. 37–54); RBC = red blood cells (n.v. 4.200–6.000); Hb = hemoglobin (n.v. 11.5–18); WBC = white blood cells (n.v. 5–10); PT = platelets (n.v. 150–300).

adriamycin dose ranged from 40 to 70 mg/m^2, depending on clinical protocol requirements. The drug was administered by i.v. push in 1–2 min and heparinized venous blood samples were collected before drug administration and serially at intervals of 5, 10, 15, 30, 45, 60, 75, 90, 120, 180, 240 and 360 min after treatment.

Preparation of plasma and blood cells

Plasma was separated from the total cellular fraction by centrifuging 2 ml of blood at 6000 *g* for 15 min immediately after collection. In 3 patients (Nos. 4, 13 and 14) the different blood cell types (RBC, WBC and PT) were separated. For this purpose, 9 ml of blood were collected at 5, 30 and 120 min after drug injection and mixed thoroughly with 1 ml trisodium citrate (3.8%) to prevent coagulation. The blood was spun in a Beckman centrifuge at 400 *g* for 10 min; the supernatant was platelet-rich plasma (PRP), the lower layer contained RBC, and WBC were between the two phases. A suspension of washed platelets was prepared by centrifuging PRP at 6000 *g* for 10 min and resuspending the platelet pellet in ice-cold 0.154 M NaCl. This washing procedure was repeated twice.

WBC were prepared by treating the buffy coat with 1% ammonium oxalate (1:9 v/v) for 10 min to remove the remaining red cells. Like PT, both RBC and WBC were then washed twice with ice-cold 0.154 M NaCl. Cell counts were made in a Bürker hematocytometer, diluting 20 μl blood with 4 ml formal citrate for RBC, with 0.38 ml Türk solution for WBC and with 1.98 ml 1% ammonium oxalate for PT by the Unopette method and counting by phase contrast microscopy.

Drug assay

AM was measured in plasma and blood cells after extraction and deproteinization with *n*-butyl alcohol, according to the fluorimetric procedure described by Finkel *et al.* for daunomycin [15]. Readings were taken in an Aminco Bowman Spectrofluorometer at 470 mμ excitation and 569 mμ emission. From plasma, RBC, WBC and PT recovery was 90% and sensitivity 0.025 μg/ml. The drug concentrations are actually AM equivalents, as *n*-butyl alcohol extracts both the primary compound and its metabolic derivatives. However, previous studies by this group [16] indicated that most of the fluorescence measured at early times after drug injection was accounted for by unchanged drug and its reduced metabolite adriamycinol, the only metabolite contributing to AM's cytotoxic activity [17]. Therefore, total fluorescence may be taken as an estimate of the active form of adriamycin.

The percentage of AM in the plasma or the total cellular fraction was determined by relating the levels of drug in plasma or blood cells of 1 ml whole blood to the volume occupied by the two fractions, according to the hematocrit value.

To determine the relationship between Ht values and the percentage of drug in plasma or blood cells, the least squares method was used, the significance of the regression being tested by the *F*-test. Areas under the concentration versus time curves (AUC) were calculated by trapezoidal integration.

RESULTS

The histories of patients submitted to AM treatment for ovarian, uterine, lung or breast cancer and their hematological parameters are set out in Table 1.

Broad variations in Ht within ranges of normal (49%) and some definitely low values (23%) are accompanied by parallel modifications in erythrocyte count and Hb values, WBC and PT counts being independent of the HT.

Measurements of AM fluorescence in the whole blood of these patients, treated i.v. with drug doses ranging from 40 to 70 mg/m^2 (Table 2), indicated that the blood concentrations of AM increased in proportion to the dose. When AM was measured separately in plasma and blood cells from the same patients, the sum of the amounts of drug found in the two fractions of 1 ml blood was the same as that measured in 1 ml whole blood. However, considering the percentage distribution to plasma and the blood cell fraction, it appears that the amount of AM in each patient's plasma and blood cells is related to the volume occupied in blood by the cellular component, as expressed by Ht, the drug concentrations rising in blood cells and declining in plasma as the Ht increases (Table 3). For instance, the patient with an Ht of 23% has a limited drug content in blood cells, the average percentage concentration in time being only 18%, with plasma levels more than 3 times higher. In contrast, the patient with the Ht of 49% accumulates AM in the cellular fraction more than in plasma, although the difference in the average percentage drug amount is larger than expected on the basis of the cellular volume. From the absolute drug concentrations in the two blood fractions of these patients (Fig. 1) it can be seen that, with equivalent AM levels in whole blood, broad variations occur in the cellular and plasma component as a function of

Table 2. Levels of AM (μg/ml) in whole blood of patients after graded drug doses

Time after treatment (min)	AM dose (mg/m² i.v.) to different patients*										
	40(1)	40(2)	40(3)	40(12)	60(6)	60(9)	70(5)	70(7)	70(8)	70(10)	70(11)
5	2.41	3.46	4.73	2.83	4.41	5.97	7.38	7.13	5.36	7.43	3.53
10	0.98	1.80	—	1.25	2.00	2.11	3.43	3.29	2.57	2.87	1.87
15	—	—	1.54	0.66	0.99	—	1.74	1.98	1.44	1.63	0.94
30	0.22	0.33	0.53	0.41	0.50	0.47	0.81	1.10	0.69	0.61	0.66
45	—	—	0.27	0.27	0.42	0.36	0.42	0.67	0.33	0.44	0.34
60	0.12	0.180	—	0.23	0.34	0.29	0.24	0.50	0.30	0.35	0.18
75	—	—	0.16	—	—	—	—	—	—	—	—
90	—	—	—	0.20	0.27	0.20	—	—	0.20	0.21	0.12
120	0.11	0.11	0.12	0.19	0.25	0.21	0.15	0.31	0.20	0.16	0.10
180	—	—	—	0.18	0.21	0.19	0.16	—	0.17	0.17	—
240	0.07	0.07	0.10	—	0.20	0.18	0.18	0.22	0.10	0.13	—
360	—	—	—	—	0.17	0.17	—	0.09	0.09	0.15	0.12
AUC (μg/ml × min)	43.5	55.6	85.5	52.9	112.8	114.9	108.9	159.4	82.7	116.3	76.7

In parentheses patient number, as listed in Table 1.
*Patients Nos. 4, 13 and 14 are not included in this table.

Table 3. Mean percentages of AM in plasma and blood cells from 1 ml whole blood of patients with increasing Ht values.

Ht value	Patient	*Mean per cent (minimum and maximum values)	
%	No.	Plasma	Blood cells
23	12	81.9 (76.9–85.3)	18.1 (14.7–23.1)
28	1	62.0 (59.5–69.1)	38.0 (30.9–40.5)
31	6	70.7 (52.1–79.7)	29.3 (20.3–47.9)
33	10	68.0 (52.6–78.6)	32.0 (21.4–47.4)
36	2	49.3 (38.3–60.0)	50.7 (40.0–61.7)
37	7	57.2 (47.9–66.3)	42.8 (33.7–52.1)
38	9	64.4 (49.6–75.4)	35.6 (24.6–50.4)
39	8	61.0 (54.3–67.3)	39.0 (32.7–45.7)
40	11	69.7 (60.3–77.5)	30.3 (22.5–39.7)
42	5	46.9 (21.1–65.4)	53.1 (34.6–78.9)
49	3	37.7 (34.2–44.3)	62.3 (55.7–66.7)

*These values were calculated by averaging the relative concentrations of AM found in the plasma and cellular fractions of 1 ml blood at the various sampling times indicated in Table 2 and Materials and Methods.
The sum of the relative concentrations in the two blood fractions was assumed to be 100%.

their relative volumes. For instance, the pattern of AM distribution to plasma and blood cells is very similar when the cellular volume represents about 50% of the total blood volume (patient with Ht 49%).

Plotting the AM concentration in plasma, expressed as the average percentage concentration, against the Ht value for each patient, a highly significant linear correlation is found ($r^2 = 0.79$, $n = 11$) (Fig. 2). AM concentrations measured separately in the erythrocyte, leukocyte and platelet fractions of three cancer patients, expressed per cell or unit volume (μ^3), are reported in Table 4. A single leukocyte, on account of its larger volume and the presence of a nucleus, accumulates much more AM than a RBC or a PT; erythrocytes, although they are ten times larger than PT, take up AM to a surprisingly similar extent. However, on the basis of the AM concentration expressed per μ^3 of each cell type, drug levels in blood cells are higher than in the plasma fraction; this in-

Table 4. Distribution of AM per cell or unit volume of each blood cell type in cancer patients Nos. 4 (40 mg/m² i.v.), 13 (70 mg/m² i.v.) and 14 (70 mg/m² i.v.)

Time after treatment (min)	Patient No.	Plasma $\mu g \times 10^{-11}/\mu^3$	RBC		WBC		PT	
			$\mu g \times 10^{-11}$/cell	$\mu g \times 10^{-11}/\mu^3$	$\mu g \times 10^{-11}$/cell	$\mu g \times 10^{-11}/\mu^3$	$\mu g \times 10^{-11}$/cell	$\mu g \times 10^{-11}/\mu^3$
5	4	0.36	29.3	0.33	710	0.40	14.1	1.88
	13	0.13	81.3	0.90	1032	0.58	41.3	5.81
	14	0.27	75.7	0.84	1215	0.69	55.6	7.41
30	4	0.03	1.6	0.02	254	0.14	2.9	0.39
	13	0.03	5.4	0.06	303	0.17	7.3	0.97
	14	0.03	5.2	0.06	410	0.23	3.6	0.48
120	4	0.014	0.8	0.009	260	0.15	1.8	0.24
	13	0.013	2.4	0.027	284	0.16	4.3	0.57
	14	0.011	2.0	0.022	707	0.40	2.4	0.32

The concentration of AM per cell was calculated by relating the drug measurement in each cell type to the actual cell count in each patient. RBC and pt volume was considered 90 μ^3 [26] and 7.5 μ^3 [27] respectively. For WBC the volume was considered 1766 μ^3 as calculated by the formula of the sphere $4/3\ \pi r^3$, assuming mean diameters to be 15 μ [28].

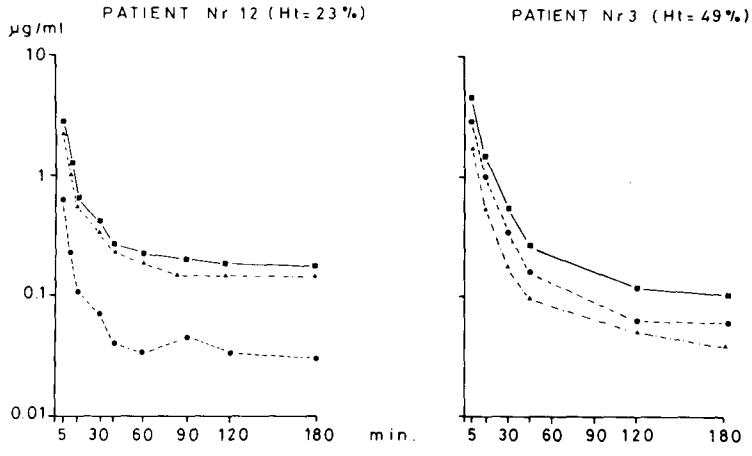

Fig. 1. Disappearance of AM from plasma and blood cells from 1 ml *whole blood of* 2 *patients with different Ht values after* 40 *mg/m*^{2} *i.v.* ■—■, *whole blood;* ▲–·–·▲, *plasma from* 1 ml *blood;* ●----●, *blood cells from* 1 ml *blood.*

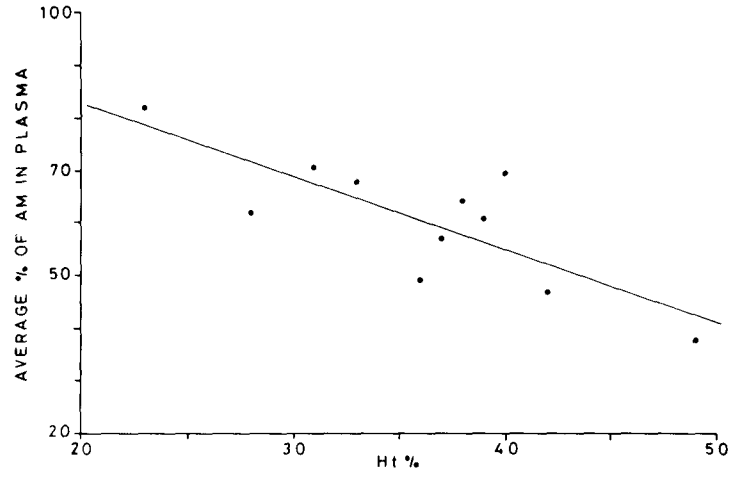

Fig. 2. Correlation between Ht values and amount of AM in the plasma fraction as expressed by average percentage concentration in plasma of 1 ml *whole blood* ($r^2 = 0.79$, $n = 11$, $P < 0.01$).

dicates that the cellular blood component accumulates AM against a gradient. In particular, PT concentrate the drug 6–10 times more than RBC and WBC. It is also worth noting that the AM content of the various blood cells of patients 13 and 14, given a dose of 70 mg/m^2, is much higher, expressed either per cell or unit volume, than in the respective cells of patient 4 treated with 40 mg/m^2, indicating that in this dose range no saturation of blood cells occurs; in plasma, however, no difference in the AM concentration between the 40 and 70 mg/m^2 doses is observable.

A further aspect of these results which deserves consideration is the disappearance rate of AM from RBC, WBC and PT. Although the limited time points are too few for calculation of half-life, they indicate that AM disappears more rapidly from RBC than PT and particularly than from WBC, where the presence of nuclear binding sites may prolong AM retention. At 30 and 120 min after treatment no substantial modifications in drug amount are observed in WBC, either per cell or per μ^3, whereas in RBC the amount of compound left at 120 min is less than half that at 30 min.

DISCUSSION

The findings described indicate that different doses of AM given to cancer patients result in drug concentrations in the whole blood showing a clear tendency to increase in proportion to the dose (Table 2). However, the relative amounts of drug in the plasma and cell fractions per ml of blood of each patient is a function of the number of cells/ml, i.e., of the volume occupied by the total cellular component (Ht). This is pertinent because cancer patients, who receive multiple cytotoxic therapy, frequently show lower than normal

hematocrit values (Table 1). As observed in rats bearing Walker 256 carcinosarcoma[1] with hematocrit values lower than in normal animals, in humans with cancer, too, when the number of blood cells/ml is reduced, the amount of AM present in the cell fraction of 1 ml blood is reduced and more of the drug is found in the plasma fraction. The relatively higher AM content in the plasma fraction parallels the lower content in the cells of 1 ml blood as a function of hematocrit values (Fig. 1).

It has been widely reported that the growth of a tumor affects the distribution of drugs and therefore their plasma levels and biological properties, by impairing hepatic metabolism [18–20] and renal excretion [21], reducing the albumin/globulin ratio in plasma [22] and protein binding [23, 24]. Modified drug accumulation in blood cells, resulting in different percentages of drug present in plasma, could be a further factor accounting for differences in the therapeutic effects of drugs in tumor-bearing subjects.

The finding that AM, in agreement with results in animals [1, 12], accumulates in blood cells, particularly PT, to a marked extent and against a gradient, as expressed by the drug concentration per unit volume, and persists for longer in WBC and PT than RBC, suggests that the various blood cells have different roles in either transport, storage or metabolism of the compound, as already shown for Vinca alkaloids in PT [4, 6].

That metabolism of AM may occur in the cellular fraction of the blood and to different extents, depending on the cell type, is suggested by Huffman and Bachur's report [7] on the AM analog, daunomycin, which is reduced to daunorubicinol by preparations of human blood cells, particularly lymphocytes, the targets of its therapeutic activity. Moreover, conversion of AM to free radicals in intact red blood cells has been demonstrated by Henderson *et al.* [25]. Whatever the role of blood cells, though, these results in our opinion suggest that changes in the total blood cell count as expressed by different Ht values must considerably influence the amounts of AM found in the plasma and cellular fraction, and that plasma concentrations are not wholly representative of drug availability in blood. These speculations acquire particular relevance in the presence of drugs, showing a plasma/cell distribution considerably different from 1. This is the case of the adriamycin analog, daunomycin, which has been shown to accumulate in RBC twice as much as in plasma [12]. In this case, therefore, changes in the volume of the cellular fraction could significantly alter the relative distribution of the drug in plasma and blood cells. This point could be worth investigating in future studies.

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