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Influence of Tumor on Adriamycin Concentration in Blood Cells

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Summary. *The relative distribution of adriamycin to plasma and blood cells after IV injection of 10 mg/kg was investigated in CD rats bearing intramuscular 256 Walker carcinosarcomas 15 days old. The drug was measured by a fluorimetric procedure and the amount of unchanged compound was separated from metabolites and quantitated by means of a TLC scanning fluorescence technique. In the presence of a tumor much lower hematocrit values are found, with marked anemia and thrombocytopenia associated with leukocytosis. These modified hematologic parameters account for an altered pattern of drug distribution. The low number of blood cells per milliliter results in a smaller amount of drug being present in the cellular fraction, so that more of the compound (even twice as much) is made available in plasma. Changes in adriamycin concentrations per unit volume or cell of each cell type are inversely related to changes in their relative number per milliliter. The only cell fraction where the drug increase per cell or cubic micrometer does not compensate the marked reduction in cell count observed in the presence of tumor is the platelet fraction, in which adriamycin amounts are 25% or less of those observed in the blood of normal rats, indicating that these blood cells become saturated in tumor-bearing animals.*

Introduction

In a preceding paper describing the differential distribution of the anthracycline antibiotics adriamycin (AM) and daunomycin (DM) to blood components of rats [T. Colombo et al., submitted for publication], it was reported that drug distribution to the various blood cell types was quantitatively important, accounting for more than 50% of the total drug concentration present in blood, and that these drugs accumulated in blood cells, particularly platelets, against a gradient. However, these studies were conducted in normal animals with normal hematocrit (HT), whereas anthracycline antibiotics are utilized in tumor-bearing subjects, whose HT values are frequently low. It is thus reasonable to assume that when the cellular component accounts for a smaller volume the amounts of drugs in blood cells and plasma may change, and this may have a biological significance.

The pattern of distribution of AM to plasma and blood cells was therefore investigated in rats bearing intramuscular Walker 256 carcinosarcoma, where anemia, leukocytosis, and thrombocytopenia are associated with the tumor growth.

Materials and Methods

Animals, Tumor, and Treatment

CD male rats (150 ± 10 g) obtained from Charles River, Italy were used for these experiments. The Walker 256 carcinosarcoma, maintained in solid form by SC serial passages in this strain every 2 weeks, was transplanted IM at a suspension of 10^6 viable cells. Tumor-bearing rats were used 15 days after tumor transplant, when the primary tumor weighed 31 ± 7 g and marked anemia, leukocytosis, and thrombocytopenia were present (see Table 1). AM was injected IV in a dose of 10 mg/kg and blood samples were obtained up to 120 min after drug administration by intracardiac puncture from open-chest animals under ether anesthesia.

Preparation of Plasma and Blood Cells

Blood was collected in disposable plastic syringes containing 1 part of trisodium citrate (3.8%) for 9 parts of blood to prevent coagulation; the samples were mixed thoroughly and centrifuged in a plastic tube at 1800 rpm for 10 min. The supernatant phase was rich in platelets (PRP); red cells (RBC) accumulated in the lower layer; and white cells (WBC) between the two phases.

Suspensions of washed *platelets* (PT) were prepared by centrifuging PRP at 4000 rpm for 10 min and resuspending the platelet button in the same volume of isotonic saline. This washing procedure

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Table 1. Hematological parameters of CD rats 15 days after transplantation of IM Walker 256 carcinosarcoma

	HT % ± SE	RBC/mm³ × 10³ ± SE	WBC/mm³ × 10³ ± SE	PT/mm³ × 10³ ± SE
Controls	43 ± 2	5413 ± 178	8.3 ± 0.5	817.7 ± 41.6
Walker	18.7 ± 1.2[a]	2368 ± 174[a]	24.7 ± 3.5*	174.7 ± 23.1*

* $p < 0.01$ (Student's t-test)

was repeated twice. White cells were prepared by treating the WBC buffy coat with 1% ammonium oxalate to remove the remaining red cells. Like the platelets, both white and red cell fractions were then washed twice with isotonic saline. Cell counting was performed in a Bürker hemocytometer, 20 µl blood being diluted 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. A Unopette was used and phase-contrast microscopy was applied.

Drug Assay

AM was measured in plasma, RBC, WBC, and PT after *n*-butyl alcohol extraction according to the fluorimetric procedure described by Finkel [6]. Recovery was 90% ± 3% and absolute sensitivity was 0.025 µg/ml. The drug concentrations are actually AM equivalents, as *n*-butyl alcohol extracted both the primary compound and its metabolic derivatives. The amounts of unmetabolized AM present in the total drug fluorescence, measured by a scanning fluorescence technique [2, 14], indicate that the unchanged compound amounts to 100% in all blood fractions, small amounts of reduced metabolite (adriamycinol) being present only in plasma and RBC at 60 min after drug injection.

Results

The hematologic picture, expressed by the blood cell count, of CD rats bearing 15-day-old IM Walker carcinoma differs consistently from that in normal animals (Table 1). In the presence of tumor, marked anemia and thrombocytopenia are associated with high leukocytosis, and the total cellular fraction accounts for a much lower volume (lower HT).

When AM is injected IV in a dose of 10 mg/kg to Walker-bearing rats, higher AM concentrations are detectable in the blood, particularly in the plasma fraction, at 1 and 120 min after treatment, and the pattern of drug distribution to plasma and blood cells reflects the altered hematologic parameters (Table 2). In the blood of tumor-bearing rats the plasma and WBC fractions account for larger volumes, because of lower HT and leukocytosis, and accumulate AM more than in control blood on a percentage basis (in WBC the phenomenon is less evident). The drug amount in the PT fraction, whose count in Walker rats is 25% that of normal animals, appears markedly reduced.

However, if the concentration of AM is expressed per cell or unit volume of each cell type (Table 3), in controls a single leukocyte, on account of its larger volume, accumulates AM much more than an erythrocyte or a platelet, but the concentration per unit volume is higher in PT. Compared with controls, much more compound is present in RBC of Walker-bearing rats and the same trend is observed, although less markedly, for PT of tumoral animals. The opposite is true for WBC, where much lower AM concentrations per cell or per unit volume are detectable in the blood of Walker rats. These differences in AM accumulation in blood cells in the presence of tumors markedly reduce the differences in drug distribution to the various blood fractions expected on the basis of the cell count. For instance, in spite of large differences in RBC count between controls and tumor-bearing rats, the percentage of AM present in this blood fraction appears very similar in both groups (Table 2), and in fact the higher drug concentration per unit volume of Walker rat erythrocytes (approximately twice as high as in controls) masks the lower RBC count in the presence of tumor (half as much).

Discussion

The findings described in the present report indicate that the hematologic pathology, in particular lower HT values, usually associated with the presence of a tumor greatly modifies the relative distribution of AM to plasma and blood cells of Walker-bearing rats. When the number of blood cells per milliliter is reduced the amount of drug present in the cell fraction is also reduced and more of the compound (even twice as much) is present in the plasma fraction.

Since the drug activity is usually ascribed to its unbound form, it is reasonable to assume that the level reached in plasma is a major pharmacokinetic determinant of drug response. Therefore it could be postulated that the presence of a tumor, by reducing the blood cell fraction in blood and making more AM available in plasma, could enhance the response to treatment. As regards the increase in AM concentration at later times after administration (120 min) in the blood of Walker rats, one

Table 2. Percentage distribution of AM[a] in plasma and blood cells from 1 ml whole blood in Walker bearing rats (W) compared with controls (C)

Time after treatment (min)	Whole blood (μg/ml ± SE)		Plasma %		RBC %		WBC %		PT %	
	C	W	C	W	C	W	C	W	C	W
1	11.6 ± 1.1	18.4 ± 0.7[c]	37.7	58.8	49.8	38.9	4.2	4.4	3.2	0.9
15	2.2 ± 0.3	2.2 ± 0.1	49.3	52.3	32.6	40.3	15.4	10.6	7.7	1.2[c]
30	1.3 ± 0.2	1.5 ± 0.1	39.4	50.2	38.6	43.0	11.8	9.4	14.2	1.8[c]
60	0.8 ± 0.02	0.8 ± 0.1	34.1	43.0	36.6	31.1	16.8	19.6	11.0	2.6[b]
120	0.6 ± 0.001	1.2 ± 0.1[c]	24.6	62.8[c]	40.0	27.7	14.8	n.d.	12.3	< 0.5[c]

[a] CD rats received 10 mg AM/kg IV
[b] $p < 0.05$ vs controls (Student's t-test)
[c] $p < 0.01$ vs controls (Student's t-test)

Table 3. Concentration of AM per cell or unit volume of each blood cell type in Walker-bearing rats (W) compared with controls (C)

Time after treatment (min)	RBC				WBC				PT			
	μg × 10^{-11}/cell ± SE		μg × 10^{-11}/μm^3 ± SE		μg × 10^{-11}/cell ± SE		μg × 10^{-11}/μm^3 ± SE		μg × 10^{-11}/cell ± SE		μg × 10^{-11}/μm^3 ± SE	
	C	W	C	W	C	W	C	W	C	W	C	W
1	123 ± 30	187 ± 25	2.2 ± 0.5	3.4 ± 0.45	6633 ± 917	4400 ± 615	3.7 ± 0.5	2.5 ± 0.35	44 ± 10.4	114.5[c] ± 10	8.1 ± 1.9	21.2[c] ± 1.85
15	14.0 ± 2.5	33[b] ± 6	0.3 ± 0.05	0.6[b] ± 0.11	5200 ± 1016	1710[b] ± 318	2.9 ± 0.6	0.97[b] ± 0.18	18 ± 2.3	19.6 ± 4.4	3.3 ± 0.4	3.6 ± 0.81
30	9.0 ± 1.5	27[c] ± 4	0.2 ± 0.03	0.49[c] ± 0.07	1770 ± 325	360[c] ± 30	1.0 ± 0.2	0.20[c] ± 0.02	6.3 ± 3.1	8.4 ± 1.1	1.1 ± 0.5	1.56 ± 0.2
60	5.2 ± 0.6	11 ± 4	0.09 ± 0.01	0.2 ± 0.07	1950 ± 653	590 ± 96	1.1 ± 0.4	0.33 ± 0.05	8.6 ± 2.0	16.1 ± 7	1.6 ± 0.3	2.98 ± 1.3
120	3.7 ± 0.5	26[b] ± 5	0.07 ± 0.01	0.47[b] ± 0.09	1330 ± 407	n.d.	0.7 ± 0.2	n.d.	8.1 ± 0.8	< 1	1.5 ± 0.2	< 0.19
AUC at 120 min (μg × 10^{-11}/cell or μm^3 × min)	1612 ± 162	3625[c] ± 83	30.4 ± 3.0	65.7[c] ± 1.5	190906[a] ± 6246	72545[a, c] ± 3464	107[a, c] ± 3.5	41[a] ± 2.0	840[a] ± 183	1516[a] ± 256	153[a] ± 33.9	280[a] ± 47.4

[a] AUC at 60 min
[b] $p < 0.05$ vs controls (Student's t-test)
[c] $p < 0.01$ vs controls (Student's t-test)
RBC volume was considered to be 55 μm^3 [5] and PT volume 5.4 μm^3 [12]
WBC volume was considered to be 1766 μm^3, calculated by the formula for a sphere $4/3\,\pi r^3$ with assumed mean diameter of 15 μm (observed under the microscope with a microcytometer)
The concentration of AM per cell was calculated by relating the drug measurement in each type to the actual cell count in each rat at each time

explanation might be the rapid saturation [4] of the tumor compartment, which, amounting to about 10% of the animal's weight, probably has a profound influence on the relative distribution of drugs in the body.

It has been widely reported that the presence of tumor affects the pharmacokinetics of drugs, and therefore their plasma levels and biological properties by impairing hepatic metabolism [10, 13, 15] and renal excretion [3], thus reducing the albumin/globulin ratio in plasma [7] and protein binding [1, 11]. Variations in plasma drug levels resulting from modified accumulation in blood cells could be a further factor leading to altered therapeutic effects of drugs in tumor-bearing subjects, and must be taken into account when drug doses are selected.

The differences between AM distribution to plasma and blood cells in controls and tumor-bearing animals would be even greater if the drug concentration per cubic micrometer were constant in both groups; however, the fact that the differences in drug concentrations in each cell type are inversely related to changes in cell number per milliliter of blood somewhat reduces the significance in terms of drug distribution to the various blood fractions of

alterations in cell count, sometimes to a minimal level, as in the case of RBC. It would be interesting to investigate whether this process also takes place with other compounds.

This finding is difficult to interpret without postulating a compensation mechanism that does not permit broad fluctuations in drug distribution because of altered cell count and is made possible by the fact that blood cells do not seem to be saturated in normal conditions.

The only compartment where the drug increase per cell or cubic micrometer does not compensate the marked reduction in cell count observed in the presence of tumor is the PT fraction, in which the amount of AM is 25% or less of that observed in the blood of normal rats, indicating that PT become saturated in tumor-bearing animals.

If blood cells, particularly PT, are vehicles of AM and DM to targets or in some way mediators of their pharmacologic activity, as suggested previously [T. Colombo et al., submitted for publication] and as already envisaged for vinka alkaloids [8], changes in cell counts, and especially in the percentage accumulation of these drugs in each fraction, could further affect the response to treatment.

Another important point regarding AM distribution to blood components in tumor-bearing animals is the possibility that the compound is metabolized in blood cells. It has been reported that the AM analog DM is metabolized to daunorubicinol by each cell type of human blood, particularly by preparations of lymphocytes and leukocytes [9], the targets of its therapeutic effect. If this were also the case for AM, changes in drug amount in blood cells could alter metabolic processes in the blood, which might be related to its pharmacologic activity. In our opinion, the results presented here underline the importance of checking hematologic parameters carefully when studying drug distribution. Studies are now in progress to investigate whether hematologic pathology also alters the relative amounts of AM present in plasma and blood cells in humans.

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