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Cellular Uptake, Cytotoxicity, and Transport Kinetics of Anthracyclines in Human Sensitive and Multidrug-Resistant K562 Cells

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ABSTRACT. Multidrug resistance in tumor cells is often associated with the presence of an ~170 kDa plasma membrane glycoprotein (Pgp) that acts as a drug-efflux pump and decreases intracellular antitumor drug concentration. We measured the uptake of seven anthracyclines (daunorubicin, doxorubicin, 4′-epi-doxorubicin, 4′-deoxy-doxorubicin, iododoxorubicin, 3′-(3-methoxymorpholino)-doxorubicin (FCE23762) and 4-demethoxy-daunorubicin) into K562 cells sensitive and resistant (K562/DNR) to daunorubicin. The K562/DNR subline expresses Pgp at the membrane surface, whereas its sensitive counterpart does not. Laser flow cytometry was used to quantitate intracellular anthracycline content. Uptake of daunorubicin, doxorubicin, 4′-epi-doxorubicin, and 4′-deoxy-doxorubicin was minimal in the K562/DNR subline as compared to their uptake in sensitive cells. On the contrary, iododoxorubicin, FCE23762, and 4-demethoxy-daunorubicin accumulate to nearly the same extent into sensitive and resistant K562 cells. Growth inhibition data indicated that the resistance factor for iododoxorubicin, FCE23762, and 4-demethoxy-daunorubicin is markedly decreased as compared to the other drugs. Fluorescence measurements were carried out to determine the kinetic parameters associated with the influx and efflux of the drugs into and out of K562 cells. Kinetic data indicated that iododoxorubicin, FCE23762, and 4-demethoxy-daunorubicin are not actively rejected from resistant cells, suggesting that they are poor substrates for Pgp-mediated transport. This observation is related to their ability to overcome the multidrug-resistant phenotype of K562/DNR cells *in vitro*. BIOCHEM PHARMACOL 51;10:1341–1348, 1996.

KEY WORDS. anthracyclines; multidrug resistance; P-glycoprotein; transport kinetics; K562 cells; flow cytometry

MDR§ is one of the major obstacles to cancer chemotherapy. MDR cells are cross-resistant to a wide range of structurally unrelated cytotoxic compounds, such as anthracyclines and *Vinca* alcaloids [1, 2]. In most cell lines, MDR is associated with the presence of a 170 kDa plasma membrane Pgp encoded in humans by the MDR1 gene [3]. It is a member of the ABC superfamily of transport systems [4], which includes transport proteins such as the cystic fibrosis transmembrane conductance regulator or the MHC class II-linked peptide transporters [5]. It has been demonstrated that overexpression of Pgp in MDR cells results in a decreased accumulation of antitumour drugs associated with drug efflux against the concentration gradient [6]. An ATPase activity was shown to be associated with drug transport [7], but little is known about the mechanism underlying drug efflux and the way Pgp handles drugs with totally different structures. Two main approaches to modulate Pgp-related MDR have been proposed. The first is the use of chemosensitizing agents to reverse resistance in MDR cell lines [8]. Verapamil, a calcium channel blocker, and cyclosporin A are the most often mentioned and have been shown to effectively reverse MDR *in vitro* [9–11]. However, the clinical use of chemosensitizers remains questionable and the identification of more active, but less toxic, compounds is needed [8, 12, 13]. Second, some antitumour anthracyclines and related agents retaining activity against doxorubicin-resistant MDR cell lines have been identified [14, 15]. This property has been explained in terms of an improved cellular accumulation in resistant cells [16]. The role of Pgp in this modified uptake can only be assessed by a kinetic study of the uptake and efflux of drugs in the presence of sensitive and resistant cells. We address this question for a series of anthracyclines in sensitive and resistant human chronic myelogenous leukemia K562 cells. FCM was used to determine intracellular accumulation of anthracyclines, whereas transport kinetics into the cells were measured by taking advantage of the natural fluores-

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§ *Abbreviations:* ABC, ATP-binding cassette; FCE 23762, 3′-(3-methoxymorpholino)-doxorubicin; FCM, flow cytometry; K562/DNR, daunorubicin-resistant K562 cells; MDR, multidrug resistance; MHC, major histocompatibility complex; Pgp, P-glycoprotein; RF, resistance factor.

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cence of anthracyclines, as recently described [17, 18]. Together with cytotoxicity data, our measurements provide evidence that anthracyclines that are able to reverse the MDR phenotype, accumulate to the same extent in sensitive and resistant cells as a consequence of the absence of active efflux out of resistant cells.

MATERIALS AND METHODS

Drugs and Chemicals

Doxorubicin, 4′-epi-doxorubicin, 4′-deoxy-doxorubicin, 4′-deoxy-4′-iodo-doxorubicin (iododoxorubicin), FCE 23762, and 4-demethoxy-daunorubicin were obtained from Pharmacia-Farmitalia (Milan, Italy). Daunorubicin, Hepes KCl, and Triton X-100 reduced were Sigma products (St. Louis, MO, U.S.A.), glucose and NaCl were Merck products, and $MgCl_2$ and $CaCl_2$ were purchased from Janssen-Chimica (Beerse, Belgium) and UCB-Vel (Leuven, Belgium), respectively. Trypan blue was supplied by Gibco BRL.

Cell Lines

Human chronic myelogenous leukemia cell lines K562 and K562/DNR (daunorubicin-resistant) were obtained from Dr. J. P. Marie (Hôtel-Dieu, Paris, France). They were grown in RPMI 1640 medium (Gibco BRL) supplemented with 10% calf serum (Eurobio, Les Ulis, France), 2% L-glutamine (Gibco BRL), and 1% antibiotic/antimycotic solution (Gibco BRL) at 37°C in a humidified atmosphere of 5% CO_2. To maintain the resistance phenotype, K562/DNR cells were cultured in the presence of daunorubicin (1 μM) for 1 week every 2 months. All experiments with K562/DNR cells were carried out at least 1 week after the interruption of culture in the presence of daunorubicin.

Flow Cytometry

10^6 cells/mL were incubated for 3 hr with 1 μM of the different anthracyclines in RPMI 1640 medium at 37°C. They were then washed twice in PBS and resuspended in 500 μl of PBS. Cell fluorescence was measured on an EPICS®-PROFILE II cytometer (Coulter) using EPICS cytologic software; 10,000 cells were counted in each case by the cytometer. Each experiment was repeated at least 4 times.

Kinetics of Drug Accumulation

Spectrofluorimetry was used to measure the kinetics of uptake of drugs into cells [17, 18]. Anthracycline fluorescence is only quenched after drug intercalation between the base pairs of DNA, and passage through the cell membrane is the rate-limiting step during the drug uptake process [17]. 10^6 cells were suspended in 1 mL glucose-containing Hepes buffer (9.5 mM Hepes pH 7.2, 132 mM NaCl, 3.5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 5 mM glucose) at 37°C under continuous stirring. 10 μL of a 10^{-4} M anthracycline stock solution were then added to obtain a 1 μM final concentration. The fluorescence decrease was measured as a function of time with an SLM Aminco® 8000C spectrofluorometer. The fluorescence excitation wavelength was 470 nm for daunorubicin, doxorubicin, 4′-epi-doxorubicin, 4′-deoxy-doxorubicin, iododoxorubicin, and 4-demethoxy-daunorubicin and 500 nm for FCE 23762. Fluorescence emission was monitored at 590 nm for daunorubicin, doxorubicin, 4′-epi-doxorubicin, 4′-deoxy-doxorubicin, iododoxorubicin, and FCE 23762 and at 570 nm for 4-demethoxy-daunorubicin. After 1–3 hr incubation (depending on the anthracycline used), drug uptake reached a steady state (the fluorescence intensity, F_n, was constant). Triton X-100 reduced (0.05% final concentration) was then added to permeabilize the cell membranes. The fluorescence intensity reached a new value, F_N, characteristic of the equilibrium state. The effect of Triton X-100 on anthracycline fluorescence in the absence of cells was measured as a control and taken into account to calculate the value of F_N. The achievement of steady state was ascertained by the fact that for sensitive K562 cells $F_n = F_N$. Kinetic parameters associated with anthracycline transport into K562 cells were calculated as previously described [17, 18]. Briefly, the initial rate of uptake, V_+, is given by

$$V_+ = (dF/dt)_{t=0} \cdot C_T/F_0, \quad (1)$$

where $(dF/dt)_{t=0}$ is the slope of the tangent to the fluorescence curve (dF/dt) at $t = 0$, C_T the total concentration of drug added (1 μM) and F_o the fluorescence intensity at $t = 0$. On the other hand,

$$V_+ = k_+^{pHe} \cdot n \cdot C_T, \quad (2)$$

where k_+^{pHe} is the influx coefficient at a given external pH (in this case 7.2) and n the cell concentration (10^9 cells/L). The concentration of drug intercalated between the DNA base pairs at the steady state is

$$C_n = C_T \cdot [(F_0 - F_n)/F_0]. \quad (3)$$

At the equilibrium state, after addition of Triton, the concentration of drug intercalated between the base pairs is

$$C_N = C_T \cdot [(F_0 - F_N)/F_0]. \quad (4)$$

At the steady state, the extracellular free drug concentration C_e *is*

$$C_e = C_T - C_n, \quad (5)$$

at the equilibrium state, the free drug concentration C_E is

$$C_E = C_T - C_N. \quad (6)$$

The free drug concentration in the cytosol at the steady state (C_i) can be calculated, considering that, in the present work, the external pH (pHe) is equal to the internal pH (pH_i) of the cytosol = 7.2 [18]. Under these conditions,

$$C_i = (C_E/C_N) \cdot C_n. \quad (7)$$

Knowledge of C_i and C_e allows the calculation of the active efflux coefficient, $(k_-^{7.2})_a$, associated with the activity of Pgp in resistant cells. Indeed, at the steady state, the influx rate and the efflux rate are equal:

$$(V_+)_s = (V_-)_s. \quad (8)$$

$(V_-)_s$ is the sum of a passive and an active efflux:

$$(V_-)_s = (V_-)_{s,p} + (V_-)_{s,a}. \quad (9)$$

In consequence

$$k_+^{7.2} \cdot C_e = (k_-^{7.2})_p \cdot C_i + (k_-^{7.2})_a \cdot C_i, \quad (10)$$

where $(k_-^{7.2})_p$ is the passive efflux coefficient. Since

$$(k_-^{7.2})_p = k_+^{7.2}, \quad (11)$$

it follows that

$$(k_-^{7.2})_a = k_+^{7.2} \cdot [(C_e/C_i) - 1)]. \quad (12)$$

Cytotoxicity of Anthracyclines

Cells (10^5 in 1 ml of RPMI 1640 cultures medium) were incubated in 24-well multidishes for 72 hr in the presence of various anthracycline concentrations. Cell viability was assessed using Trypan blue exclusion. Viable cells in each dish were counted by microscopic analysis to determine cell growth. The ID_{50} was determined (100% cell growth accounted for cells incubated without drug). For each anthracycline, the ID_{50} for resistant cells was divided by the ID_{50} for sensitive cells to determine the RF. Each experiment was repeated 3 times.

RESULTS

Cellular Accumulation of Drugs at the Steady State

FCM was used to quantitate the cellular accumulation of different anthracyclines (Fig. 1) in K562 and K562/DNR cells at the steady state. Recorded data are displayed as histograms of cell number vs fluorescence intensity (Fig. 2A to 2G). A typical fluorescence histogram of cells incubated without any drug is also displayed (Fig. 2H). FCM measurements were carried out after 3 hr of incubation; this delay was needed to reach the steady state in the case of doxorubicin, 4′-epi-doxorubicin, and 4′-deoxy-doxorubicin. For the other compounds, a 1-hr incubation provided the same fluorescence patterns as those obtained after 3 hr. The peak of fluorescence distribution of daunorubicin, doxorubicin, 4′-epi-doxorubicin, and 4′-deoxy-doxorubicin is significatively shifted to the left when incubated in the presence of K562/DNR cells as compared to sensitive cells (Fig. 2A–2D). Clearly, their cellular accumulation is weaker in the K562/DNR resistant subline than in sensitive K562 parental cells. In contrast, iododoxorubicin, FCE 23762, and 4-demethoxy-daunorubicin fluorescence histograms are only slightly shifted for K562/DNR cells as compared to K562 cells (Fig. 2E–2G), indicating that these drugs accumulate almost to the same extent in both cell lines.

KINETICS OF DRUG TRANSPORT

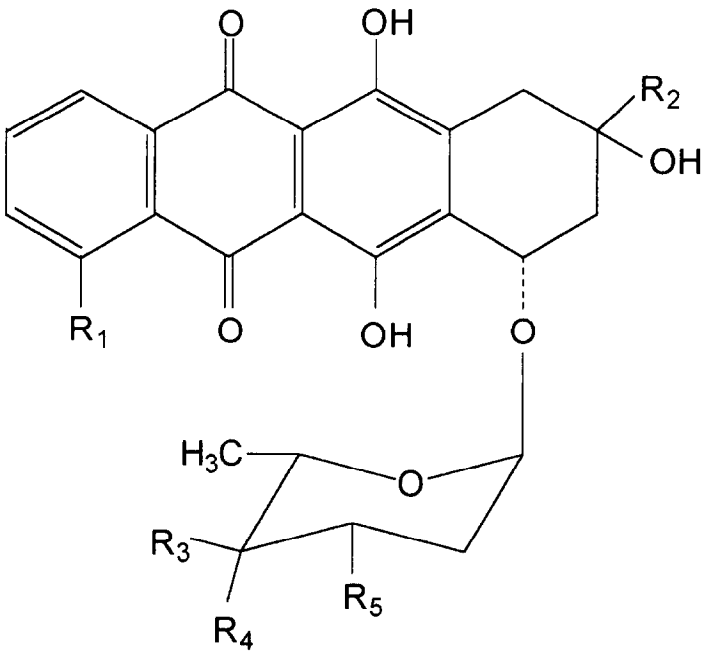
FCM only gives indications about the net accumulation of drugs in the cells at the steady state. To determine the kinetic parameters associated with the transport of the different drugs into the cells, we used a fluorometric method previously developed [17, 18]. The uptake of the different anthracyclines by sensitive and resistant cells (10^6 cells/mL) at 37°C and at pH 7.2 was measured at an initial added drug concentration of 1 μM (C_T). In each case, the initial rate of uptake (V_+), the concentration of drug intercalated between the base pairs of the nucleus at the steady state (C_n) and at the equilibrium state (C_N), free in the cytosol at the steady state (C_i) and free in the extracellular medium at the steady state (C_e) and at the equilibrium state (C_E) were calculated as described in Materials and Methods. These data allow the calculation for each drug of the influx and efflux coefficient values. Data for sensitive and resistant cells are shown in Table 1. For each compound, influx ($k_+^{7.2}$) and total efflux coefficients ($k_-^{7.2}$) are of the same magnitude for sensitive cells. On the contrary, total efflux coefficients for resistant cells are nearly twice as great as influx coefficients in the case of daunorubicin, doxorubicin, 4′-epi-doxorubicin, and 4′-deoxy-doxorubicin. Obviously, 50% of the total efflux concerns an active process (evaluated by $(k_-^{7.2})_a$) expelling drugs out of the cells. Importantly, the contribution of this active efflux does not exist in resistant cells for iododoxorubicin, FCE 23762, and 4-demethoxy-daunorubicin, which accumulate to the same extent in sensitive and resistant cells.

Cytotoxicity of Anthracyclines

ID_{50} and RF values for the different anthracyclines measured in K562 and K562/DNR cells are listed in Table 2. RF values are nearly equivalent in the case of daunorubicin, doxorubicin, and 4′-deoxy-doxorubicin. K562/DNR cells display the strongest resistance towards 4′-epi-doxorubicin. For the other derivatives (iododoxorubicin, FCE 23762, 4-demethoxy-daunorubicin) the resistance factor is, by comparison, very weak. It is noteworthy that the ID_{50} of these three anthracyclines is weaker than the ID_{50} of the other anthracyclines in sensitive cells. FCE 23762 is the derivative for which the resistance of K562/DNR cells is the most markedly reduced.

DISCUSSION

Although several mechanisms have been proposed to be responsible for the decreased drug accumulation observed in many MDR cell lines [19], it is generally accepted that it is the result of Pgp activity removing drugs from the cells in



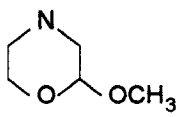
Compound	R_1	R_2	R_3	R_4	R_5
Daunorubicin	OCH_3	$COCH_3$	H	OH	NH_2
Doxorubicin	OCH_3	$COCH_2OH$	H	OH	NH_2
4'-epi-doxorubicin	OCH_3	$COCH_2OH$	OH	H	NH_2
4'-deoxy-doxorubicin	OCH_3	$COCH_2OH$	H	H	NH_2
iododoxorubicin	OCH_3	$COCH_2OH$	H	I	NH_2
FCE 23762	OCH_3	$COCH_2OH$	H	OH	
4-demethoxy-daunorubicin	H	$COCH_3$	H	OH	NH_2

FIG. 1. Anthracycline structures.

an ATP-dependent manner. In the present work, we correlate the uptake of several anthracyclines with their *in vitro* cytotoxicity and their susceptibility to be rejected out of resistant cells *via* an active process associated with this activity. It has been routinely established by FCM on cells incubated with an antiPgp monoclonal antibody (JSB-1, MRK16) coupled to an FITC-labeled secondary antibody that, contrary to sensitive cells, the K562-resistant subline expresses Pgp at a high rate at the membrane surface [20, 21]. We measured the accumulation of seven anthracycline derivatives in sensitive and resistant K562 cells. Three accumulate nearly to the same extent in both cell lines: iododoxorubicin, FCE 23762, and 4-demethoxy-daunorubicin. Total cellular fluorescence measured by FCM did not

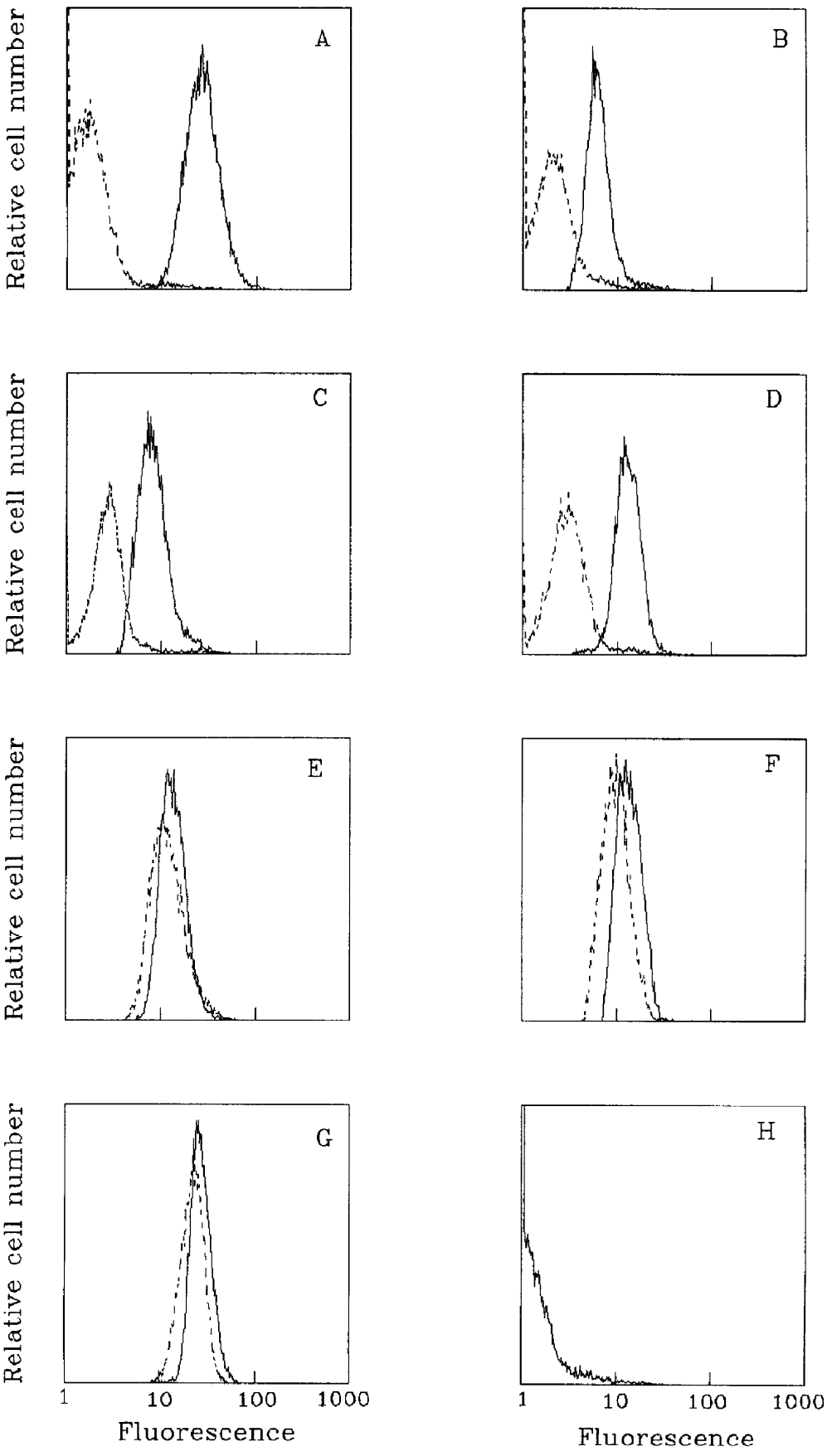


FIG. 2. Cellular accumulation of anthracyclines. Fluorescence intensity histograms were obtained by flow cytometry after incubation of K562 (—) and K562/DNR (---) cells (10^6 cells/mL) with different anthracyclines (1 μM) at 37°C. A, daunorubicin; B, doxorubicin; C, 4′-epi-doxorubicin; D, 4′-deoxy-doxorubicin; E, iododoxorubicin; F, FCE 23762; G, 4-demethoxy-daunorubicin; H, K562 sensitive cells without drugs (profile was the same for resistant cells). Fluorescence intensity is displayed on a log scale. A total of 10,000 cells were counted for each histogram. Experiments were repeated 4 times and gave essentially the same profiles as the ones shown here.

TABLE 1. Kinetics of anthracycline transport into K562 (S) and K562/DNR (R) cells

Drug		$k_+^{7.2} \times 10^{12}$	$k_-^{7.2} \times 10^{12}$	$(k_-^{7.2})_a \times 10^{12}$
		l/(cells · s)		
Daunorubicin	S	4.3 ± 0.2	4.4 ± 0.4	–
	R	3.9 ± 0.3	7.8 ± 1.1*	3.9 ± 0.6
Doxorubicin	S	2.8 ± 0.4	2.8 ± 0.4	–
	R	3 ± 0.05	5.8 ± 0.4*	2.8 ± 0.4
4′-Epi-doxorubicin	S	3.2 ± 0.1	3.2 ± 0.1	–
	R	3.3 ± 0.2	6.8 ± 1*	3.5 ± 1
4′-Deoxy-doxorubicin	S	3.5 ± 0.2	3.8 ± 0.05	–
	R	3.8 ± 0.1	8 ± 0.5*	4.2 ± 0.4
Iododoxorubicin	S	14.5 ± 0.6	14.5 ± 0.6	–
	R	10.7 ± 0.8	10.6 ± 1	–
FCE 23762	S	3.9 ± 0.36	3.4 ± 0.5	–
	R	3.3 ± 0.6	3.6 ± 0.7	–
4-Demethoxy-daunorubicin	S	7.7 ± 0.7	7.7 ± 0.7	–
	R	6.8 ± 0.3	6.9 ± 0.3	–

1 μM of drug was added at $t = 0$ to 10^6 cells in 1 mL of glucose-containing Hepes buffer (pH 7.2, 37°C) and fluorescence decay was followed as a function of time. Kinetic parameters were calculated as described in Materials and Methods. Each experiment was repeated at least 6 times. $k_+^{7.2}$, influx coefficient; $k_-^{7.2}$, total efflux coefficient; $(k_-^{7.2})_a$, active efflux coefficient (mean ± SD). *$k_-^{7.2}$ significantly different from $k_+^{7.2}$ ($P < 0.001$).

reflect the cellular drug content because anthracycline fluorescence is known to be quenched after intercalation between the base pairs of the DNA [17, 22]. Consequently, FCM does not detect the fraction of drug mainly implicated in cell death (i.e. the fraction interacting with DNA). Total cellular fluorescence of a drug at the steady state is, however, in good correlation with its cytotoxicity [23, 24] and is generally used to gain rapid information on the ability of pharmacological agents to reverse MDR [20, 25]. Our measurements confirm this because there is a close correlation between anthracycline accumulation measured by FCM and the cytotoxicity of anthracyclines in K562 cells, as indicated in Table 2. Weak RF factors for iododoxorubicin, FCE 23762, and 4-demethoxy-daunorubicin in K562 cells are in agreement with other studies describing the effectiveness of these drugs in other MDR cell lines: human small-cell lung cancer and murine mammary tumour (iododoxorubicin) [16], human lymphoblastic leukemia and human myeloid leukemia (4-demethoxy-daunorubicin) [25], and murine leukemia (FCE 23762) [14]. Moreover, correlation with cytotoxicity and an improved cellular uptake has been established for iododoxorubicin and 4-demethoxy-daunorubicin [16, 25].

TABLE 2. Determination of anthracycline cytotoxicity

Drug	ID_{50} (nM) (mean ± SD) K562	K562/DNR	RF
Daunorubicin	14.8 ± 1.9	1570 ± 235	106
Doxorubicin	18.5 ± 2.4	2200 ± 320	119
4′-Epi-doxorubicin	22.4 ± 4.4	4230 ± 640	189
4′-Deoxy-doxorubicin	12 ± 3.2	1300 ± 252	108
Iododoxorubicin	6.3 ± 0.7	40 ± 6	6.3
FCE 23762	5 ± 1	12.6 ± 1.5	2.5
4-Demethoxy-daunorubicin	6 ± 0.8	43.5 ± 8	7.3

Cells were cultured for 72 hr with increasing drug concentrations. Cell viability was assessed as described in Materials and Methods. ID_{50}, drug concentration required to inhibit cell growth by 50% as compared to the control (cells cultured without anticancer drugs); RF, resistance factor; calculated by dividing the ID_{50} for resistant cells (K562/DNR) by the ID_{50} for sensitive cells (K562).

The main question now is how to explain the identical cellular uptake of some anthracyclines into an MDR subline and its parental counterpart. Slight structural differences can significantly modify the polarity of the molecule (4-demethoxy-daunorubicin, FCE 23762) or the pKa of its amine (iododoxorubicin) and increase the percentage of drug present in the neutral form at physiological pH. Because it is known that the influx of anthracyclines into cells is the result of the passive diffusion of the neutral form of the drug [18], such characteristics could be a first explanation for the improved uptake in resistant cells.

A second possibility is that these anthracyclines are not transported by Pgp. We addressed this question by using a fluorometric method allowing an easy and rapid determination of anthracycline influx and efflux kinetic parameters [17, 18]. Advantages over previous procedures include a direct measurement of the accumulation process involving a nondestructive procedure and the fact that it does not require a filtration step susceptible of modifying efflux measurements. The evaluation of the membrane transport kinetic parameters for the different anthracyclines (Table 1) shows that, at the steady state, influx and efflux of the different drugs proceed at equivalent rates in sensitive cells (compare the $k_+^{7.2}$ and $k_-^{7.2}$ values), which is normal for a passive diffusion process. In resistant cells, an active efflux component could be measured for daunorubicin, doxorubicin, 4′-epi-doxorubicin and 4′-deoxy-doxorubicin. The total efflux component for these drugs was, consequently, twice as great as that of the influx and their intracellular accumulation at the steady state was low as assessed by

FCM. On the other hand, Table 1 indicates the absence of active efflux in resistant cells for iododoxorubicin, FCE 23762, and 4-demethoxy-daunorubicin. Again, this is in correlation with FCM measurements indicating their similar accumulation in sensitive and resistant cells at the steady state. As shown previously, active efflux is an energy-dependent process [18, 26], is absent in sensitive cells, and can be reasonably associated with Pgp activity. Therefore, our data strongly suggest that iododoxorubicin, FCE 23762, and 4-demethoxy-daunorubicin are not transported by Pgp. This is confirmed by the fact that, in our system, verapamil had no influence on the initial rate of uptake of these drugs, whereas the DNR initial rate of uptake was increased in the presence of the Pgp blocker (results not shown).

$k_+^{7.2}$ values indicate that, for 4-demethoxy-daunorubicin and, especially, iododoxorubicin, influx is rapid when compared to the other anthracyclines studied. Because passage through the plasma membrane is the rate-limiting step for anthracycline uptake, this would greatly facilitate their access to DNA and other intracellular targets. Conjugated with the absence of outward transport by Pgp, this would explain their improved cytotoxicity in resistant cells (low RF, Table 2) as compared to daunorubicin, doxorubicin, and other antitumour agents to which K562/DNR cells are cross-resistant. FCE 23762 has the weakest resistance factor. Contrary to iododoxorubicin and 4-demethoxy-daunorubicin, this can only be explained by the absence of outward transport in resistant cells. Indeed, influx proceeds at nearly the same rate for FCE 23762 and the drugs to which K562/DNR cells are cross-resistant (see Table 1, $k_+^{7.2}$ values).

Our work clearly shows that compounds that are poor substrates for Pgp are efficient cytostatic agents against MDR cells. This had been previously suggested after studies on iododoxorubicin [27] and two other anthracycline analogs (aclacinomycin A and Ro 31-3294) [28], but no direct evaluation of active efflux had been carried out. At the present time, it is difficult to establish a correlation between the absence of anthracycline interaction with Pgp and structural requirements. This would require a better knowledge of the way Pgp handles its substrates and of the active site(s) of the protein responsible for antitumour agent transport. It would, then, become possible to design new agents devoid of cross-resistance on a rational basis. From a clinical point of view, however, compounds such as iododoxorubicin, 4-demethoxy-daunorubicin, and FCE 23762, the latter a representative of the morpholinyl-substituted anthracycline family, are candidates for future evaluations in the treatment of Pgp-associated MDR tumour unresponsive to classically employed anticancer agents.

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