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THE EFFECT OF CROWN ETHERS, TETRAALKYLAMMONIUM SALTS, AND POLYOXYETHYLENE AMPHIPHILES ON PIRARUBICIN INCORPORATION IN K562 RESISTANT CELLS

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Abstract—The basic distinguishing feature of all cells expressing functional P-glycoprotein-multidrug resistance (P-gp-MDR) is a decrease in steady-state accumulation drug levels as compared to drug-sensitive controls. In an attempt to identify mechanism(s) by which MDR can be circumvented, we examined the cellular accumulation, in resistant cells, of 4′-*O*-tetrahydropyranyl-doxorubicin (pirarubicin) alone and in conjunction with various molecules belonging to three different classes: the crown ethers, the tetraalkylammonium salts, and the polyoxyethylene amphiphiles. The present study was performed using a spectrofluorometric method which enabled us to follow the uptake and release of fluorescent molecules by living cells while the cells were being incubated with the drug. Erythroleukemia K562 cell lines were used. Our data show that the compounds of these three completely different classes were able to increase the incorporation of pirarubicin provided they had a minimum degree of lipophilicity. Study of the growth inhibitory activity of these compounds revealed that cross-resistance to the tetraalkyl ammonium salt increased with the lipophilicity and was equal to 58 for tetraoctylammonium salt, the most lipophilic compound of this series. This demonstrates that neither the presence of a positive charge nor an aromatic moiety is required for MDR recognition.

Key words: multidrug resistance; doxorubicin; pirarubicin accumulation; chemosensitizer

MDR† is frequently associated with decreased drug accumulation resulting from enhanced drug efflux. This is correlated with the presence of a membrane protein, the P-gp, that pumps a wide variety of drugs out of cells, thereby reducing their intracellular concentration and, thus, their toxicity [1, 2].

The MDR phenotype can be overcome to various extents by treatment of cells with chemosensitizers or resistance-modifying agents, which increase intracellular drug accumulation specifically in MDR cells [3, 4]. The ability to reverse drug resistance is, in fact, a relatively common property of lipophilic drugs [5–9]. Although the mechanisms by which chemosensitizers modulate MDR are poorly understood, it is thought that most are either substrates for P-gp and/or inhibitors of its activity [10–12].

It has been proposed that the minimum set of structural and functional features required for modulator binding to P-gp is two planar aromatic domains and a tertiary amine nitrogen atom [13, 14]. More recently, however, Dellinger *et al.* [15] have postulated that an aromatic moiety and a critical degree of lipophilicity are required for MDR recognition for simple cytotoxic organic cations. On the other hand, Ichikawa *et al.* [16] have shown that the accumulation of basic dyes was lower in MDR KB cells than in the parent line, whereas the accumulation of acidic and neutral dyes was similar in both cell lines.

It has often been reported that most of the agents that overcome MDR and most anticancer drugs transported by P-gp are hydrophobic and cationic, and that neutral or anionic chemicals may not interact with P-gp [11]. However, anticancer drugs and agents that overcome MDR are not necessarily cationic: for example, progesterone interacts with P-gp and enhances the accumulation of vinblastine in MDR cells [17]; cyclosporin, a cyclic hydrophobic undecapeptide that strongly binds Ca^{2+} and Mg^{2+} [18], is able to reverse MDR [19] and to be transported by P-gp [20]; hydroxyrubicin, a fully neutral derivative of adriamycin, can be actively pumped out by P-gp [21]; and nonionic detergents are able to reverse MDR [8, 22, 23].

Mobile ionophores, such as valinomycin, nonactin, nigericin, monensin, calcimycin, and lasalocid, as well as cyclosporin, inhibit the P-gp-mediated efflux of drug [24–26]. We then wondered if synthetic macroring-containing oxygen atoms, such as 18-crown-6 ether [27], could inhibit the P-gp-mediated efflux of antitumor drugs such as the anthracyclines. The 18-crown-6 ether proved to be inefficient, although some more lipophilic analogs of this compound, such as decyl-18-crown-6 ether, were able to inhibit the efflux. We then examined whether or not the presence of the crown was a required structural set. Finally, we showed that simple quaternary ammonium salts having a minimum lipophilicity were also very good P-gp inhibitors.

The 33 compounds used in this study can be divided into three classes: (1) the crown ethers, (2) the tetraalkyl ammonium salts, and (3) the polyoxyethylene amphiphiles. We tested (1) their ability to increase pirarubicin incorporation in K562 doxorubicin-resistant cells, (2) the growth inhibitory activity of these compounds, and (3) the ability of octylammonium to sensitize resistant cells to pirarubicin.

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† Abbreviations: MDR, multidrug resistance; RF, resistance factor; P-gp, P-glycoprotein; THP; 4′-*O*-tetrahydropyranyl.

MATERIALS AND METHODS

Culture and cytotoxicity assays

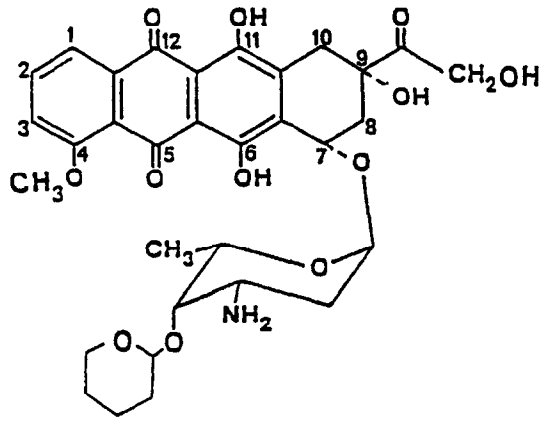
Anthracycline-sensitive and -resistant erythroleukemia K562 cells were a gift from Dr. Tapiero (Departement de Pharmacologie Cellulaire, ICIG, 94800 Villejiuf, France). They were grown in RPMI (Flow) medium supplemented with L-glutamine and 10% FCS at 37°C in a humidified atmosphere of 95% air and 5% CO_2 [19]. Cultures initiated at a density of 10^5 cells/mL grew exponentially to 8–10 × 10^5 cells/mL in 3 days. For the spectrofluorometric assays, the culture was initiated at 5 × 10^5 cells/mL and cells used 24 hr later to have cells in the exponential growth phase; they then numbered approximately 8 × 10^5 cells/mL. The sensitivity of the tumor cells to the different molecules was investigated by measuring the growth of drug-sensitive and drug-resistant cells in the presence of increasing concentrations of these agents. Cells at a density of 10^5 cells/mL were incubated for 3 days in the culture medium containing the test agents. Their effects were expressed as IC_{50}, the drug concentration that inhibits cell division by 50% after 72 hr, which were determined by plotting the percentage of cell growth inhibition *versus* the logarithm of the antitumor drug concentration; the percentage of cell growth inhibition is defined as $[(N_O - N_x)/N_O - 10^5)] \times 100$, where N_O and N_x are the numbers of cells/mL in the absence and presence of pirarubicin at concentration x, respectively. A RF was obtained by dividing the IC_{50} of resistant cells by the IC_{50} of the corresponding sensitive cells.

The ability of octylammonium to sensitize cells to pirarubicin was evaluated by treating cells with graded concentrations of pirarubicin in the presence of this agent for 3 days.

Total RNAs were prepared from frozen cells according to the CsCl-guanidinium isothiocyanate method proposed by Maniatis *et al.* [28] and adapted by Ferrandis *et al.* [29]. The transcript level of the MDR1 gene was measured comparatively to that of the KB-8-5 cell line, which shows an arbitrary expression of 30 a.u. [30]. Our K562 resistant cells exhibited an MDR1 gene transcript level of 800 a.u. (Benard and Garnier-Suillerot, unpublished data). The resistance phenomenon was stable over the period required for these experiments.

Drugs and chemicals

Purified pirarubicin (Scheme 1) was kindly provided by Laboratoire Roger Bellon (France). Concentrations were determined by diluting stock solutions to approximately 10^{-5} M and using $\epsilon 480$ = 11,500 M^{-1} cm^{-1}. Stock solutions were prepared just before use. Verapamil and compounds of the polyoxyethylene series were purchased from Sigma. Trade names of these compounds (where n is the number of ethylene oxide units) are as follows: Triton X-15 (n = 1), 15-S-3 (n = 3), Nonidet P-14 (n = 4), Triton X-45 (n = 5), X-114 (n = 8), X-100 (n = 9.6), X-102 (n = 12), X-165 (n = 16), X-207 (n = 20), X-305 (n = 30), X-405 (n = 40). The tetraalkyl ammonium salts $N^+[(CH_2)_nCH_3]_4$ (n = 1–7), $N^+[CH_3]_2[(CH_2)_nCH_3]_2$ (n = 9, 11), $N^+[CH_3]_3[(CH_2)_nCH_3]$ (n = 11, 15) were from Aldrich. The crown ether/18-crown-6, /Kriptofix 22, /dicyclohexyl-18-crown-6, /decyl-18-crown-6, /kryptofix 22DD, /krytofix-221D, /dibenzo-18-crown-6, /krytofix-5, /dicyclohexyl-24-crown-8; /dibenzo-30-crown-10 were from Merck and /benzo-18-crown-6, /N,N'dibenzyldiaza-18-crown-6 from Aldrich. All compounds were prepared as stock solutions in deionized double-distilled water or in tetrahydrofuran. All other reagents were of the highest quality available. Experiments were performed in Hepes Na^+ buffer solutions containing 20 mM Hepes buffer plus 132 mM NaCl, 3.5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 5 mM glucose at pH 7.25.



Scheme 1. Pirarubicin.

Absorption spectra were recorded on a Cary 219 spectrophotometer and fluorescence spectra on Jobin Yvon JY 3CS and Perkin Elmer LS 50B spectrofluorometers.

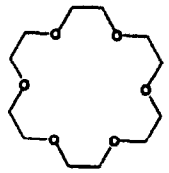
Cellular drug accumulation

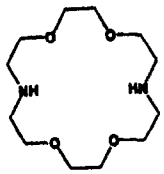
The uptake of anthracycline in cells was followed by monitoring the decrease in the fluorescence signal at 590 nm (λ_{ex} = 480 nm). This spectrofluorometric method that we have developed (previously described in [31–33]) is based on the observation that fluorescence of anthracycline is only quenched when intercalated between the base pairs of DNA. At concentrations lower than 12 μM, where the fluorescence intensity is roughly proportional to the drug concentration, the overall concentration of anthracycline intercalated between the base pairs in the nucleus can be quantitatively determined from the decrease in the fluorescence signal observed when cells are incubated with anthracycline. Using this method also makes it very easy to measure the initial rate of drug uptake by cells accurately. The sensitizer at concentration [i] is added once the steady state is reached, yielding a new steady state and a new amount of pirarubicin intercalated between the base pairs. This can be quantified using the following equation:

$$(C_n)_{Ri} = (C_n)_{Ro} + [(C_n)_S - (C_n)_{Ro}]\alpha \qquad (1)$$

where $(C_n)_S$ is the overall concentration of drug bound to the nucleus of sensitive cells and $(C_n)_{Ro}$ and $(C_n)_{Ri}$ the overall concentrations of drug bound to the nucleus of resistant cells in the absence and presence of a concentration [i] of inhibitor, respectively [34]. α is the fold increase in pirarubicin incorporation in the presence of modulator. α varies between 0 (in the absence of modulator) and 1 (when the amount of drug in resistant cells is the same as in sensitive cells).

All experiments were conducted in a 1-cm quartz cuvette containing 2 mL of buffer at 37°C. At the end of the experiment, cell viability was assessed using trypan

[1] 



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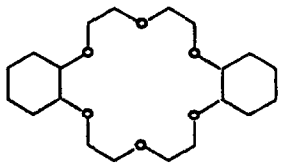
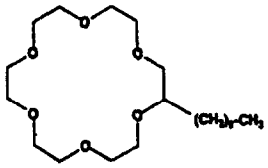
[3] 



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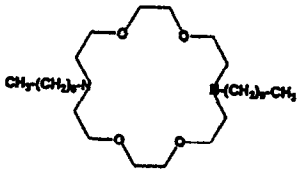
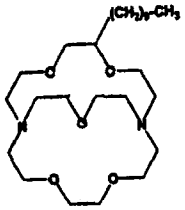
[5] 



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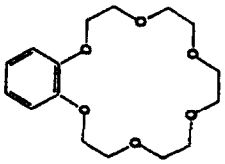
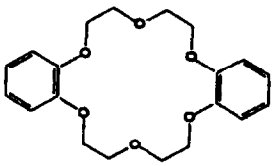
[7] 



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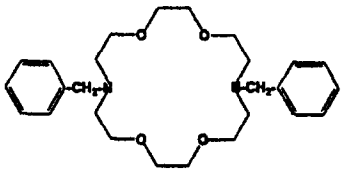
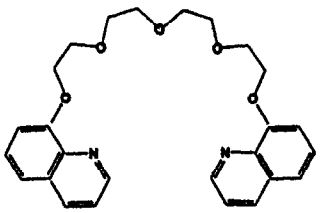
[9] 



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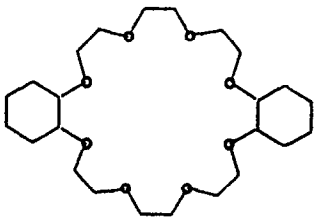
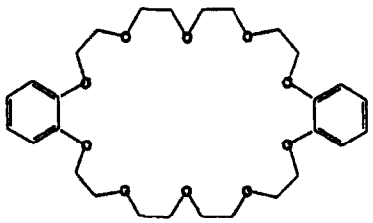
[11] 



[12]

Scheme 2. Crown ethers.

blue exclusion. The final amount of tetrahydrofuran was lower than 0.3% and did not affect cell viability.

RESULTS

Pirarubicin was used in the present study instead of the more commonly used anthracycline derivatives doxorubicin and daunorubicin because it is taken up by cells rapidly and the steady state is reached within approximately 30 min. It is, thus, very easy to follow the modification of its intracellular accumulation in the presence of various chemosensitizers monitoring its fluorescence decay [21, 31]. THP-adriamycin is a weak base with its intracellular accumulation depending on intracellular and extracellular pH values [24, 31]. We have previously determined that the cytosolic pH of our K562 cells, either sensitive or resistant, is equal to 7.25 ± 0.10 [33]. Here, we verified that the compounds used in the present study did not give rise to intracellular pH modification and subsequently that they did not modify the fluorescence signal of pirarubicin nor the intracellular accumulation of the drug in sensitive cells.

Influence of crown ethers on the uptake of pirarubicin by drug-resistant cells

The uptake of pirarubicin by drug-resistant cells was determined by continuous monitoring of fluorescence decay of the drug as it was incubated with the cells. In all the experiments, the intra- and extracellular pH were the same and equal to 7.25 ± 0.1. When 10^6 cells/mL were incubated with 1 μM of drug, the steady state was reached within approximately 30 min and the overall concentration (C_n) of drug bond to the nucleus at the steady state was then equal to 0.25 ± 0.02 μM (Fig. 1). The addition of crown ethers (numbered from (1) to (12), Scheme 2) yielded an increase in intracellular drug concentration. For each of them, the overall concentration of pirarubicin bound to the nucleus was determined at the steady state in the presence of different concentrations of crown ether. Figure 1 shows the typical plot of C_n as a function of the chemosensitizer concentration where the modulator was Kryptofix-221D (6). As can be seen, the amount of drug bound to the nucleus increased as the concentration of (6) increased. The chemosensitizer concentration required to obtain $\alpha = 0.5$ is reported in Table 1. Kryptofix-22 (2) may be considered inefficient because at 150 μM, α was still equal to 0. The efficiency of 18-crown-6 (1) is very low; for example, α was equal to only 0.15 in the presence of 20 μM. However, the other compounds tested in this series that bear additional CH_2 groups, were able to increase pirarubicin incorporation at concentrations comparable to that required for verapamil ($\alpha = 0.5$ at 1.6 μM). This is seemingly due to an increase in the lipophilicity of the molecule. The two compounds (11) and (12) with larger crown size (24 and 30 instead of 18) were less efficient.

Influence of tetraalkylammonium salts on the uptake of pirarubicin by drug-resistant cells

Strictly analogous experiments were performed with tetraalkyl ammonium salts containing aliphatic chains of different lengths. The molecule contains either four identical chains [(13) to (18)], two chains and two methyl groups [(19) and (20)], or one chain and three methyl

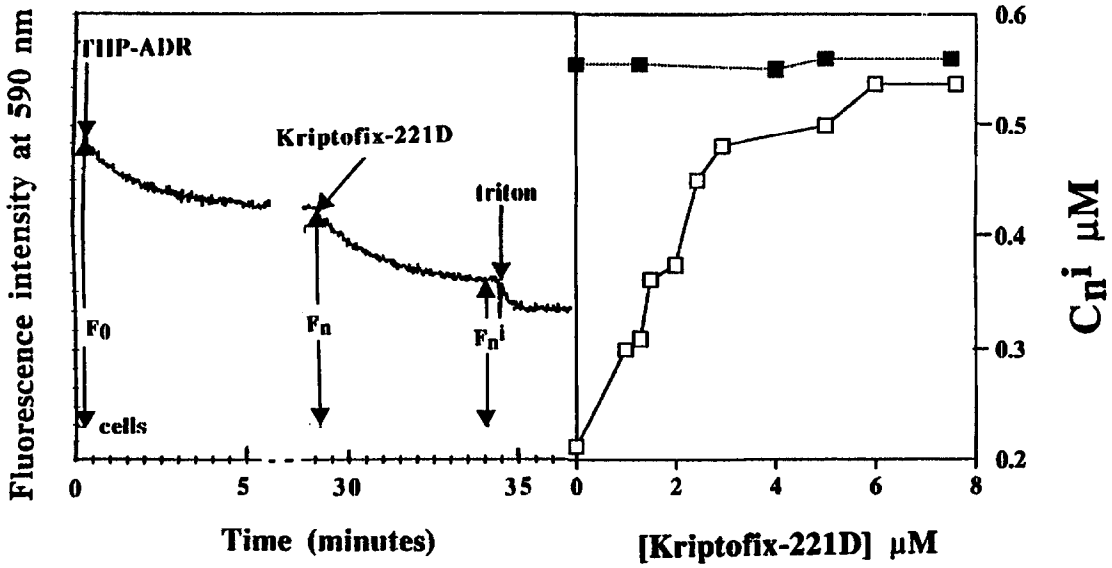
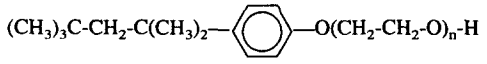


Fig. 1. Effect of Kriptofix-221D (6) on THP-adriamycin (THP-ADR) accumulation in living cells. (Left) uptake of THP-adriamycin by drug-resistant K562 cells. F, fluorescence intensity at 590 nm (λ_{ex} = 480 nm) was recorded as a function of time. Cells (2×10^6/mL) were suspended in a cuvette filled with 2 mL buffer at pH 7.25 under vigorous stirring. At $t = 0$, 20 μL of a 100-μM stock THP-adriamycin solution was added to the cells, yielding a $C_T = 1$ μM THP-adriamycin solution. The fluorescence intensity was then F_O and the concentration of drug intercalated between the base pairs in the nucleus was $C_n = C_T(F_O - F_n)/F_O$. At the steady state 2.5 μM Kriptofix-221 was added. The fluorescence of the new steady state was F_n'. When the new steady state was reached, 5 μL 0.05% Triton X-100 was added. (Right) C_n' (the overall concentration of THP-adriamycin bound to the nucleus at the steady state) has been plotted as a function of the Kriptofix-221D concentration. (■) Sensitive cells; (□) resistant cells.

Table 1. Effect of crown ethers, tetraalkylammonium salts, and polyoxyethylene amphiphiles on the short-term measurements of pirarubicin accumulation in drug-resistant K562 cells

Compounds	[i] μM* (α = 0.5)
18-Crown-6 ether derivatives	
(1)	≫100
(2)	≫100
(3)	4 ± 1
(4)	3 ± 0.8
(5)	2 ± 0.4
(6)	2 ± 0.4
(7)	≫100
(8)	15 ± 3
(9)	5 ± 1
(10)	50 ± 10
24-crown-8 ether derivative	
(11)	10 ± 2
30-crown-10 ether derivative	
(12)	12 ± 2
N^+ $[(CH2)_nCH_3]_4$	
(13) $n = 1$	≫100
(14) $n = 3$	≫100
(15) $n = 4$	≫100
(16) $n = 5$	12 ± 2
(17) $n = 6$	1 ± 0.2
(18) $n = 7$	1 ± 0.2
N^+ $[CH_3]_2[(CH_2)_nCH_3]_2$	
(19) $n = 9$	2.5 ± 0.5
(20) $n = 11$	2 ± 0.4
$N^+[CH_3]_3[(CH_2)_nCH_3]$	
(21) $n = 11$	≫100
(22) $n = 15$	≫100
	
(23) $n = 1$	35 ± 8
(24) $n = 3$	14 ± 3
(25) $n = 4$	4 ± 1
(26) $n = 5$	7 ± 2
(27) $n = 8$	10 ± 3
(28) $n = 9.6$	5 ± 1
(29) $n = 12$	7 ± 2
(30) $n = 16$	9 ± 2
(31) $n = 20$	3 ± 1
(32) $n = 30$	50 + 10
(33) $n = 40$	50 ± 10

* [i] are the chemosensitizer concentrations that cause a half-maximal increase in cellular pirarubicin accumulation. The values represent means ± SD of triplicate determinations.

groups [(21) and (22)]. The concentrations of each of these molecules required to obtain α = 0.5 are reported in Table 1. The efficiency of the molecules did not depend on the number of chains but primarily on the number of methylene groups, here the only factor modifying the lipophilicity of the molecules because all of them contain one quaternary nitrogen and four methyl group. As the number *n* of methylene groups increased, a step decrease in the concentration required to obtain α = 0.5 was observed. Thus, for the compounds with a number of methylene groups lower than or equal to 16, α = 0.5 was obtained at concentrations higher than 100 μM; when this number was higher than or equal to 17, α = 0.5 was obtained at concentrations lower than 10 μM. Here also, the efficiency of some of the molecules, i.e. (17), (18), (19), and (20), compares to that of verapamil.

Influence of Triton on the uptake of pirarubicin by drug-resistant cells

Loe and Charon have recently studied the interaction of multidrug-resistant Chinese hamster ovary cells with amphiphiles of the polyoxyethylene series [22]. They showed that MDR cells displayed collateral sensitivity to nonionic detergents, such as Triton X-100 and NP-40, and that the response of MDR cells to a series of Triton/Nonidet-type amphiphiles was very sensitive to the length of the polyethylene chains. This prompted us to investigate whether or not these compounds were able to increase pirarubicin accumulation in resistant cells. This was done as previously described for the other compounds, with the concentrations required to obtain α = 0.5 reported in Table 1. When the number *n* of ethylene oxide units increased from 4 to 20, the Triton concentrations yielding α = 0.5 were of the same order of magnitude, ranging from 3 to 10 μM. However, for the two molecules where $n < 4$ [(23) and (24)] and > 20 [(32) and (33)], the concentrations were higher (Fig. 2).

Influence of the chemosensitizers on the uptake kinetics of pirarubicin by drug-resistant cells

The initial rate of pirarubicin uptake was determined in the absence and in the presence of chemosensitizers. None of these molecules yielded a modification of uptake kinetics of pirarubicin.

Cell sensitivity to the chemosensitizers

K562 cells were tested for their sensitivity to most of the chemosensitizers, with the data reported in Table 2. Each compound is toxic to resistant cells at a concentration higher than or equal to that required to inhibit the growth of parental cells. Compounds of the crown ether family are only slightly more toxic towards sensitive cells than to resistant cells.

In the case of tetraalkyl ammonium salts, cross-resis-

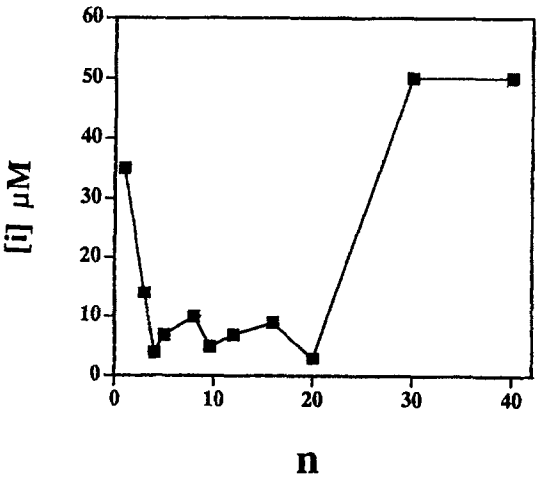


Fig. 2. Effect of polyoxyethylene amphiphiles on pirarubicin incorporation in resistant K562 cells. [i] the chemosensitizer concentration that causes a half- maximal increase in pirarubicin incorporation has been plotted as a function of the number *n* of ethylene groups.

Table 2. Cytotoxic effects of doxorubicin, pirarubicin, crown ethers, tetraalkylammonium salts, and polyoxyethylene amphiphiles on K562 sensitive and resistant cells

	IC_{50} (S)* (μM)	IC_{50} (R)* (μM)
Doxorubicin	10^{-2}	0.3
Pirarubicin	4×10^{-3}	3.2×10^{-2}
(plus 2 μM [18])		1.2×10^{-2}
(plus 2 μM verapamil)		10^{-2}
18-crown-6 ether derivatives		
(1)	6	12
(2)	2	4
(3)	6	6
(4)	2	2
(5)	2	4
$N^+ [(CH_2)_nCH_3]_4$		
(14) $n = 3$	12	12
(16) $n = 5$	0.4	4.8
(17) $n = 6$	0.4	2.8
(18) $n = 7$	0.2	12
(plus 1 μM verapamil)		2.4
$N^+ [CH_3]_2[(CH_2)_nCH_3]_2$		
(20) $n = 11$	1	5
$(CH_3)_3C\text{-}CH_2\text{-}C(CH_3)_2$—⟨○⟩—$O(CH_2\text{-}CH_2\text{-}O)_n\text{-}H$		
(25) $n = 4$	10	10
(26) $n = 5$	13	13
(27) $n = 8$	8	8
(28) $n = 9.6$	8	8
(29) $n = 12$	5	10
(30) $n = 16$	5	15
(31) $n = 20$	2	10
(32) $n = 30$	40	120
(33) $n = 40$	21	105

* IC_{50}(S) and IC_{50}(R) are the compound concentrations required to inhibit 50% of sensitive and resistant cell growth, respectively. The values represent means of triplicate determinations.

tant for the compounds with a small number of methylene groups was not observed. However, when the amount of methylene was increased, a concomitant increase in the resistance factor was noted (Table 2). In the case of tetraoctylammonium, (18), the RF was equal to 58. Verapamil was able to modify resistance and, in the presence of 2 μM, RF decreased to 12.

For the compounds of the Triton family, the two cell lines exhibited similar sensitivity to compounds containing a small number n of ethylene oxide units [(25) to (28)]. However, the compounds containing more ethylene oxide units were more toxic to sensitive than to resistant cells.

When the ability of tetraoctylammonium salt to sensitize resistant cells to pirarubicin was tested, it was observed that in the presence of 2 μM tetraoctylammonium (18), the pirarubicin resistance factor was decreased from 8 to 3 (Table 2).

DISCUSSION

Despite the great many papers published over the last 10 years concerning the molecular system that protects MDR cells against lipophilic cytotoxic drugs, very little is known. Recently, Hofsli and Nissen-Meyer have screened 26 arbitrarily chosen lipophilic drugs and shown that the ability to reverse drug resistance is, in fact, a relatively common property of lipophilic agents [9].

In this study, we have tested the ability of three different classes of compounds to increase pirarubicin accumulation in MDR K562 cells. The compounds of these three classes are able to restore pirarubicin accumulation in resistant cells, providing they have a minimum degree of lipophilicity. Because the kinetics of drug uptake is not modified by these compounds, we can infer that their kinetics of P-gp-mediated efflux is modified.

Let us, first, consider the compounds of the tetraalkylammonium series. Even in the absence of direct proof of the binding of compounds (16), (17), and (18) to P-gp, the observation that the resistance of MDR cells to these compounds can be reversed by verapamil strongly suggests that these compounds are P-gp substrates. In such a circumstance, our present data show that organic cations that do not contain a single aromatic moiety, as proposed by Dellinger *et al.* [15], but only a quaternary ammonium is recognized by MDR cells. However, a minimum degree of lipophilicity is also required and the resistance factor increases as the lipophilicity of the compound increases.

It is interesting to compare these compounds to two other classes of compounds which the K562 cell line is cross-resistant to, the anthracycline derivatives and the vinca alkaloids. The three anthracycline derivatives (doxorubicin, daunorubicin, and idarubicin) have the same amino sugar with a pK_a of deprotonation equal to 8.4 [23]. Their lipophilicity is equal to 1.2, 7.8, and 13.1 for idarubicin, daunorubicin, and doxorubicin, respectively [35]. However, their resistance factors follow the opposite order, i.e. 32, 18, and 3 for adriamycin, daunorubicin, and idarubicin, respectively [36]. Numerous other examples can be found in the literature that show that lipophilic anthracycline derivatives have a low resistance factor [37]. Vinblastine, vincristine, and vindesine have the same pK_a of deprotonation (7.4), their lipophilicity increasing from vindesine to vinblastine [38]. Concomitantly, their resistance factors decrease from vindesine to vinblastine [39]. Thus, for these two classes of drugs, the resistance factor is as low as the lipophilicity is high.

How can we explain the two apparently contradictory observations: (1) that the resistance of cells to drugs such as the anthracycline derivatives and vinca alkaloids decreases as the lipophilicity of the derivatives increases, and (2) that the resistance of cells to organic cations such as alkypyridiniums and tetraalkyl ammonium salts increases when the lipophilicity increases and that, in both cases, the standard MDR modulator verapamil reverses the resistance to these compounds.

It seems reasonable to assume that, in both cases, the cytotoxicity of a compound depends on its intracellular concentration. The intracellular accumulation of drugs depends on their kinetics of passive diffusion and their kinetics of P-glycoprotein-mediated efflux. The fact that the RF of anthracyclines is as low as the lipophilicity of the molecule is high can be explained by the observation that, for this class of molecules, the uptake kinetics are more dependent on lipophilicity than the kinetics of P-glycoprotein-mediated efflux [36]. This means that an increase in lipophilicity yields an increase in uptake kinetics and, therefore, in intracellular drug concentration. In the case of the organic cation, it is tempting to propose that the kinetics of active efflux is more dependent on lipophilicity than the uptake kinetics. In that event, an increase in molecule lipophilicity would yield an increase in the kinetics of the active efflux and, thus, a decrease in intracellular drug concentration.

The restoration of pirarubicin incorporation in MDR cells in the presence of tetraalkylammonium compounds could be due to their competing for efflux by P-gp. The efficacy is as good as the lipophilicity of the compound is high. This observation also holds for vinca alkaloid and anthracycline derivatives. We have recently shown that vinblastine, vincristine, and vindesine are able to increase pirarubicin incorporation in MDR cells with this ability increasing with a rise in lipophilicity and, therefore, a decrease in the RF value [40]. Friche *et al.* have demonstrated the *in vitro* circumvention of anthracycline resistance in Ehrlich ascites tumour cells by anthracyclines and concluded that lipophilicity is the major determinant of the ability of an analogue to increase daunorubicin accumulation [35].

For the two other classes of compounds, it is more likely that the restoration of pirarubicin incorporation is due to a modification in membrane fluidity. It has been shown that alterations in the physical state of plasma membrane lipids can influence a number of important protein-mediated processes [40]. Very recently, Dudeja *et al.* [8] have demonstrated that *in vitro* addition of active MDR-reversing surfactants decreased the lipid fluidity of the isolated crude plasma membrane of resistant cells and inactive surfactants failed to influence membrane lipid fluidity.

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