Circumvention of P-GP MDR as a Function of Anthracycline Lipophilicity and Charge

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ABSTRACT: From a number of studies it has been suggested that positive charge and degree of lipophilicity dictate, or at least influence, whether anthracyclines are recognized by the apparently clinical important mechanism of tumor cell resistance, i.e., P-gp-mediated multidrug resistance. Using a selected series of analogs in which lipophilicity and or positive charge are altered we find the following: (1) Positively-charged anthracyclines as compared to their neutral counterparts are better recognized by MDR+ cells. (2) With increasing lipophilicity charge becomes less important for MDR recognition. (3) In MDR+ cells with a resistance index to Adriamycin (ADR) of 4534, as compared to an MDR− parental line, almost all of the resistance is circumvented (resistance index = 3) with an anthracycline which does not contain a protonatable nitrogen and is highly lipophilic (partition coefficient, log p = >1.99). (4) As lipophilicity is increased to log p >1.99 and nuclear binding is decreased, anthracycline localization switches from nuclear to cytoplasmic which most likely indicates a different cytotoxic target and mechanism of action. (5) Cytoplasmically localized anthracyclines appear to distribute also in mitochondria which suggests these organelles as possible new anthracycline targets. In contrast, ADR shows no mitochondrial localization. (6) Photoaffinity analysis suggests that the highly lipophilic analogs, regardless of charge, interfere with NASV-Vp binding to P-gp. This is consistent with the idea that highly lipophilic anthracyclines act as modulators of MDR which may contribute to their mechanism of overcoming this form of resistance. The possible clinical significance of these data is that highly lipophilic anthracyclines are shown to circumvent MDR which most likely reflects their ability to localize in the cytoplasm and affect targets other than nuclear DNA, i.e., mitochondria, and to act as self modulators of MDR. Thus, a new approach to circumventing MDR and other mechanisms of resistance which involve nuclear targets is the use of active anthracyclines which are highly lipophilic and localize in the cytoplasm/mitochondria.

INTRODUCTION

P-gp-mediated multidrug resistance (MDR) appears to be a ubiquitous mechanism whereby many different types of cells recognize and resist a number of differently structured compounds (Kartner & Ling, 1989). The protein associated with the active transport of these agents out of the cell has been shown to bind most of the agents it expels (Safa et al., 1986; Safa, 1993). The question of how a single protein is able to recognize a wide variety of compounds remains unanswered.

The studies that have directly addressed this question have utilized complex compounds for structure function analysis. Results from these studies have indicated that positive charge and lipophilicity in rhodamine analogs (Lampidis et al., 1989) and other complex organic cations (Beck, 1987; Baguely et al., 1988; Kiue et al., 1990) as well as alkyl chain length in phenothiazine derivatives (Ford et al., 1989) significantly affect drug recognition by MDR+ cells. Recently, using very simple organic cations, we defined the minimal requirements for compound recognition by cells expressing P-gp-mediated MDR i.e. a single aromatic ring and an alkyl chain length ≥ 5 (log p = >−1) (Dellinger et al., 1992).

Adriamycin (ADR), a member of the anthracycline family of compounds, is widely used as an effective chemotherapeutic agent. It is believed, however, that since ADR is also recognized by P-gp MDR its clinical effectiveness is reduced (Kartner & Ling, 1989). To assess the role that charge (amination) and lipophilicity play in this form of resistance we selected to study three charged anthracyclines, Adriamycin ADR, AD288, and AD198, with increasing lipophilicity and their 3′-deaminated (uncharged) counterparts, WP159, WP546, and WP549, respectively, using MDR+ and MDR− Friend leukemic cells. Coincident with our interests in the mechanisms of anthracycline-induced cardiotoxicity, cardiac muscle and cardiac fibroblasts grown *in vitro*, which are also MDR− and MDR+ cell types, respectively, were used to analyze the intracellular localization and relative accumulation of these compounds.

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METHODS

Cell Types. Newborn rat primary cultures of cardiac-muscle cells and nonmuscle cells, which are, respectively, MDR− and MDR+ (see below), were grown on glass coverslips as previously described (Lampidis et al., 1980) and used to evaluate anthracycline cellular localization and relative accumulation. The MDR− Friend leukemic cell line (FLC) and its ADR-induced MDR+ variant (ARN 15) were obtained as previously described and used to measure the growth inhibitory potency of each of the anthracyclines listed (Tapiero et al., 1984). Since cardiac muscle cells have a very limited capacity to divide in culture, cytotoxicity assays with these cells could not be carried out. They are used in this paper, however, since they attach and spread out on glass coverslips and thus facilitate analog localization and accumulation studies.

Growth Inhibition Assays. Growth inhibition assays were performed by seeding exponentially growing FLC (MDR−) and ARN 15 (MDR+) cells at 2.0×10^4/mL in Eagle's minimal essential medium supplemented with 10% fetal bovine serum at 37 °C in 10% CO_2−air and treating each continuously with the indicated doses of anthracycline analogs. In experiments where verapamil (Vpl) was used, cells were cotreated with the test anthracyclines as indicated. At 72 h, cells excluding trypan blue were counted and survival curves were derived as previously described (Tapiero et al. 1984).

Assay of Intracellular Drug Localization and Relative Accumulation. Cardiac muscle (MDR−) and cardiac fibroblasts (MDR+) growing on coverslips, 5 days after primary culture seeding, were treated with 10 μg/mL of either ADR, WP159, AD288, WP546, AD198, or WP549 for 30 min. Cells were rinsed, and droplets of fresh medium were placed in wells of rubber dams pressed onto glass slides. After cells were rinsed, in drug-free medium, the glass surface containing the cells was placed face down in the well of the rubber dam containing the droplet of fresh drug-free medium. A seal was made between the rubber dam and the coverslip containing the cells. In this way, live cells are maintained and drug localization and accumulation can be monitored on the coverslip for prolonged periods (>24 h). Cardiac muscle and nonmuscle cell mitochondria are easy to identify by phase-contrast microscopy when growing stretched out on cover slips and can be verified by staining with Rho 123 and viewing the same field with fluorescent light, UV 540 λ.

Partition Coefficient Assays. Anthracycline analogs were suspended in 3 mL of buffer saturated with 1-octanol and then another 3 mL of 1-octanol in buffer was added. Solutions were mixed by vortex and shaken at room temperature overnight. At 24 h, samples were centrifuged (1000*g*) and the phases separated. The amount of drug in each phase was determined using standard curves constructed by plotting drug concentration as a function of measured fluorescence intensity. The working range for the standard curve was between 2×10^{-8} and 2×10^{-6} M. Coefficients were calculated by dividing the amount of drug in the 1-octanol phase by the amount found in the the buffer phase.

Photoaffinity Analysis. Photoaffinity labeling was carried out using exponentially growing ARN 15 (MDR+) cells at 5×10^5 per assay. Cells were suspended in Ca^{++}/Mg^{++}-free Dulbecco's phosphate-buffered saline containing 4% (vol/vol) dimethyl sulfoxide and 1 mM of the photoaffinity analog of verapamil, *N*-(*p*-azido-3-[^{125}I]salicil) aminomethyl-verapamil, ([^{125}I]NAS-VP) specific activity, 1100 Ci/mmol in a final volume of 0.05 mL. The cells were then preincubated for 30 min at room temperature in the absence or presence of ADR, WP159, AD288, WP546, AD198, WP549, respectively. Photoaffinity labeling was carried out as previously described (Safa, 1988). The photolabeled samples were then processed for SDS−PAGE as described previously (Safa, 1988).

RESULTS

Intracellular Localization and Accumulation of Anthracycline Analogs. The effects of charge and lipophilicity of anthracyclines on their differential accumulation in cardiac muscle and nonmuscle (fibroblasts) cells were assayed by treating 7 day old primary cultures for 30 min with each drug at the indicated doses. The anthracyclines used in this study are arranged in pairs (Figure 1) with each pair either containing or not containing a protonatable amino group on the sugar portion of the molecule. The lipophilicities vary as indicated by the log of their partition coefficients listed in Table 1. Reading the congeners from left to right and from top to bottom, they are organized from least lipophilic upper left to most lipophilic bottom right. They are further organized by listing the protonatable anthracyclines on the left-hand side of the figure and their neutral 3′-oxy counterparts directly to their right. Theoretically, the uncharged 3′-oxy congeners should be more lipophilic than their corresponding counterparts containing basic nitrogen, however, at log *p* values >1.99 these differences are not measurable.

As shown in Figure 2, drug accumulation and localization differences were noted between cardiac muscle (MDR−) and nonmuscle (MDR+) cells. Results for each compound are presented in the order of their increasing partition coefficients, i.e., ADR < WP159 < AD288 < WP546 < AD198 < WP549 (see Table 1). The least lipophilic anthracycline tested, ADR, showed differential accumulation in the cardiac muscle (CM) vs nonmuscle cardiac fibroblasts (NM) and was predominantly found in the nucleus of both cell types. For the neutral analog of ADR, WP159, the same result was obtained; however, there was less differential accumulation between CM vs NM cells. For compound AD288, there was even less differential staining between the two cell types, and mitochondrial localization of the drug was evident. With the neutral equivalent of AD288, analog WP546, much less nuclear staining was seen than with the other drugs and more diffuse cytoplasmic with some mitochondrial staining was apparent. AD198 also yielded very little or no nuclear staining but stained mitochondria as well as cytoplasmic areas both particularly and diffusely. With the neutral analog WP549, similar non-nuclear staining was seen, but the overall staining pattern in the cytoplasm and in mitochondria was somewhat more diffuse. Table 1 summarizes these observations and demonstrates a correlation between increasing lipophilicity of anthracyclines and the (1) shift from nuclear to mitochondrial/cytoplasmic intracellular staining and (2) loss of differential accumulation between MDR− (cardiac muscle) and MDR+ (cardiac fibroblast) cells.

Anthracycline Accumulation in Isolated Nuclei. In order to test whether the localization of the anthracyclines in intact

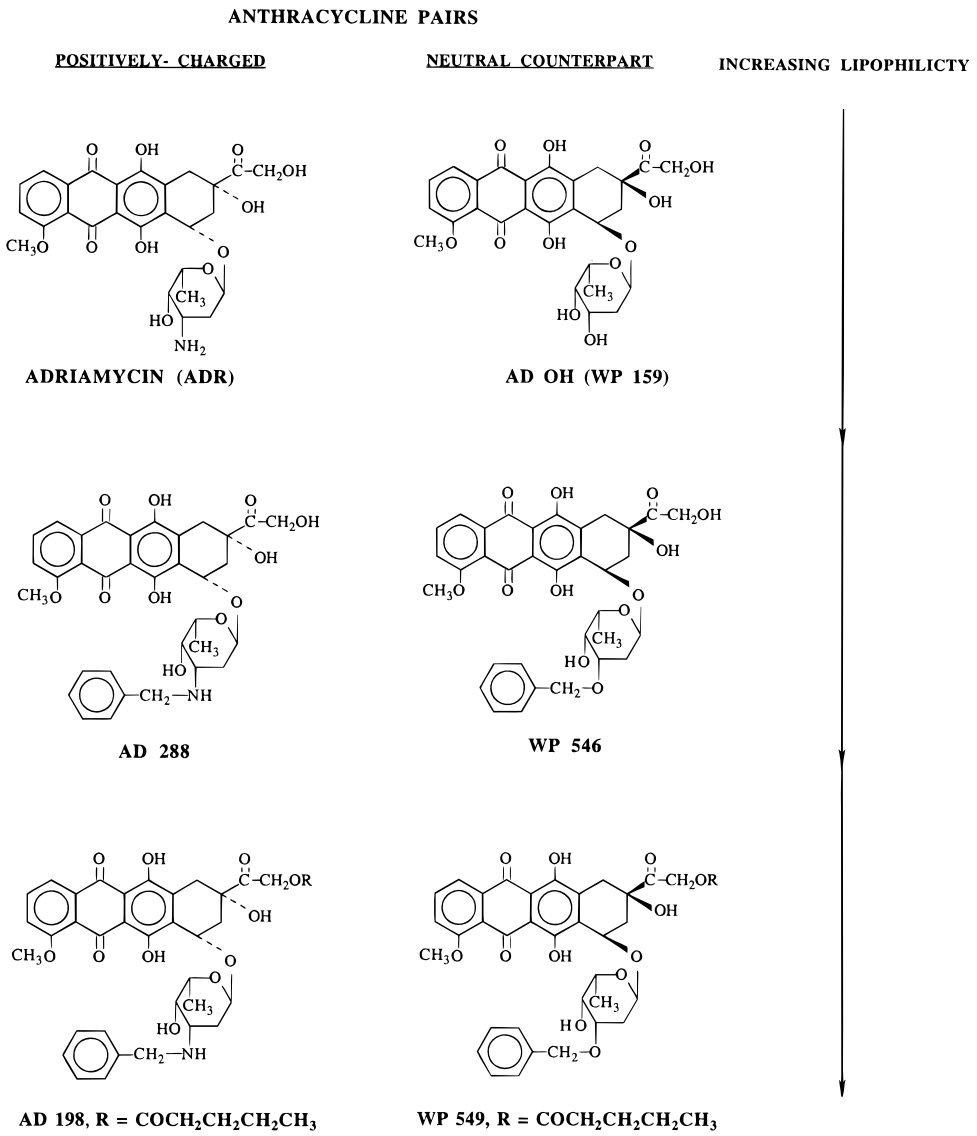

FIGURE 1: Structure of anthracycline analogs varying in charge and lipophilicity.

Table 1 [a]

drug	localization	charge	log p[b]	CM/FB[c]	(ID_{50}, [M]) MDR− (FLC)	(ID_{50}, [M]) MDR+ (ARN15)	res index[d]
ADR	nucleus	+	0.08	+++/+	6.78×10^{-9}	3.06×10^{-5}	4513
WP159	nucleus	0	1.40	+++/++	1.58×10^{-8}	6.29×10^{-6}	411
AD288	nucleus & some mito	+	>1.99	+++/++	2.53×10^{-8}	8.42×10^{-7}	33
WP546	cyto/ diffuse & mito/nuc < AD 288	0	>1.99	++/++	6.51×10^{-7}	2.90×10^{-6}	4
AD198	mito/cyto part & diffuse metachromatic	+	>1.99	++/++	2.0×10^{-8}	2.10×10^{-7}	11
WP549	diffuse (mito) < AD198 more diffuse staining in fibroblast	0	>1.99	++/+++	5.0×10^{-7}	1.56×10^{-6}	3

[a] Mito = mitochondria; cyto = cytoplasm; nuc = nucleus; part = particulate. [b] Partition Coefficient. [c] Relative fluorescence brightness in cardiac muscle cells (CM) vs cardiac fibroblasts (FB). [d] Ratio of doses yielding 50% growth inhibition in MDR+ (Adriamycin-induced)/MDR− (parental) Friend leukemic cells.

cells was affected by nuclear/DNA quenching, isolated nuclei from MDR− cells were treated with each analog, and after 30 and 120 min drugs were extracted and assayed by HPLC. The amounts of drug extracted (picomoles) at 30 and 120 min, respectively, for each of the analogs was as follows: ADR, 587, 625; WP159, 228, 206; AD288, 661, 708; WP546, 58, 71; AD198, 10, 50; and WP549, 2.5, 2.5. These results coincide with the above studies in intact cells which show that ADR, WP159, and AD288 localize in nuclei whereas very little or no nuclear staining is seen with the other three analogs and suggest that nuclear/DNA quenching does not alter the microscopic observations of nuclear/cytoplasmic localization.

Cytotoxicity Patterns of Anthracycline Analogs in MDR+ and MDR− Cell Lines. FLC (MDR−) and ARN 15 (MDR+) cell lines were treated with each of the six anthracycline analogs for 3 days, and the doses required to inhibit 50% growth (ID_{50}) for each of the compounds were determined (Table 1 and Figure 3, left panel). The results of these experiments revealed two general trends: (1)

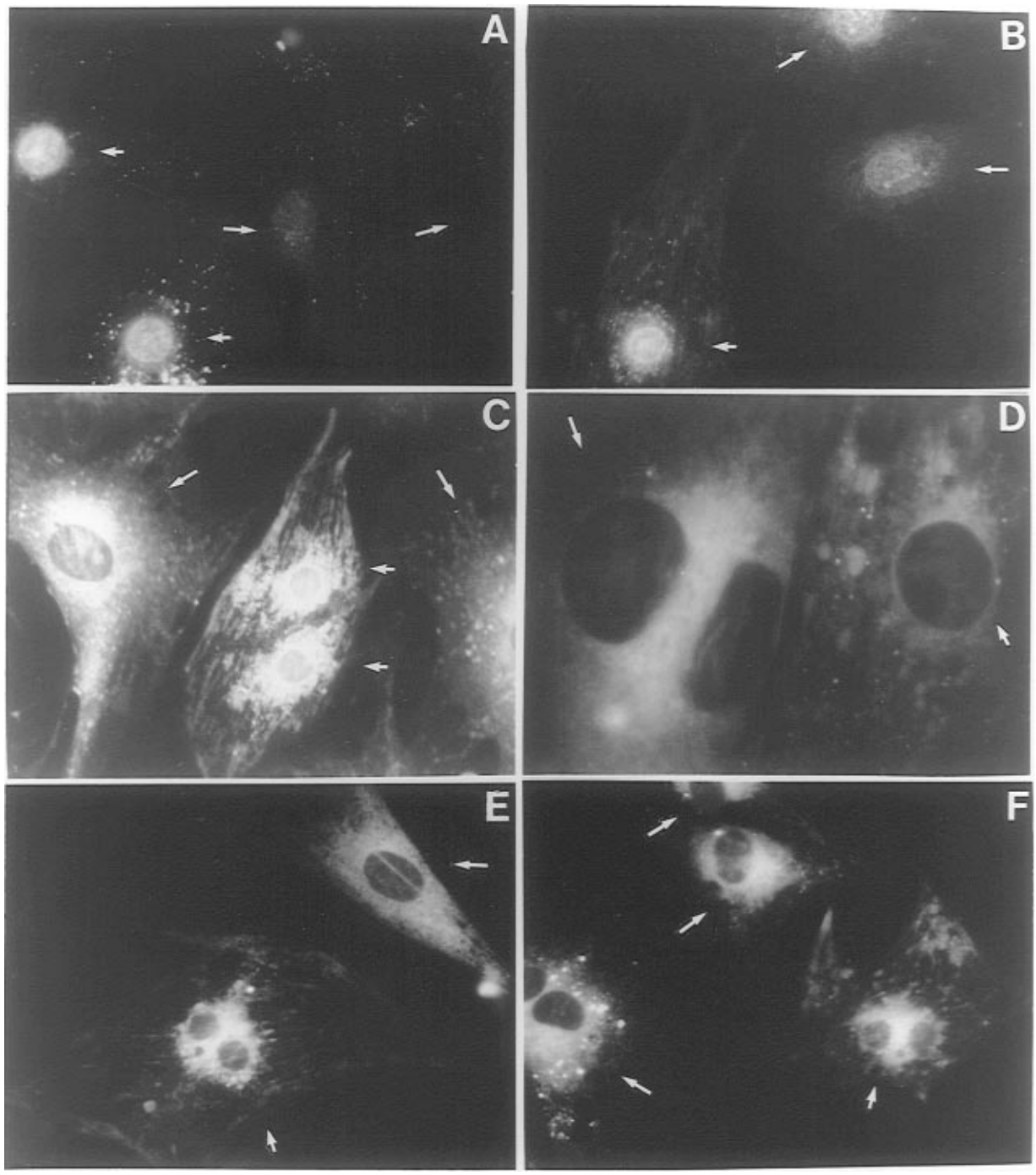

FIGURE 2: Fluorescence micrographs of cardiac muscle (MDR−, short arrow) and cardiac fibroblasts (MDR+, longer arrow) treated for 30 min with 20 μg/mL of each of the following anthracyclines rinsed three times with drug-free media and immediately observed under fluorescence microscopy, 540 λ, mag 1400: (A) ADR, (B) WP159, (C) AD288, (D) WP546, (E) AD198 and (F) WP549.

Decreased resistance indices, i.e., ID_{50} (MDR+)/ID_{50} (MDR−) of uncharged compounds as compared to their protonatable counterparts and (2) decreasing resistance indices as a function of increasing anthracycline lipophilicity. Thus, resistance indices to the highly lipophilic, neutral analogs WP546 and WP549 are markedly reduced and almost abolished as compared to the 4513 resistance index to ADR.

To better evaluate the effect that differential drug accumulation has on the cytotoxicity differences observed in these cell lines, growth inhibitory experiments were performed in the presence of the classic P-gp-mediated MDR inhibitor, verapamil (Vpl). Table 2 and Figure 3 (right panel) illustrate a relationship between the degree of resistance reversed by Vpl and the charge and lipophilicity of the anthracyclines studied. Thus, 10 μg/mL Vpl reversed resistance to the positively-charged least lipophilic anthracycline, ADR, more (98.1%) than to its more lipophilic and uncharged counterpart, WP159 (86.6%). Similarly, Vpl reversed resistance to the positively-charged, more lipophilic analog, AD288, (92.4%) than to its uncharged counterpart, WP546 (32.4%). Similar results were seen for AD198 (81.8%) versus its uncharged counterpart, WP549 (0%).

P-gp Binding of Anthracyclines by Photoaffinity Analysis. Results using the verapamil (Vpl) photoaffinity analog [^{125}I]-NAS-VP in exponentially growing MDR+ (ARN 15) Friend leukemic cells indicate that ADR at 250 μM markedly

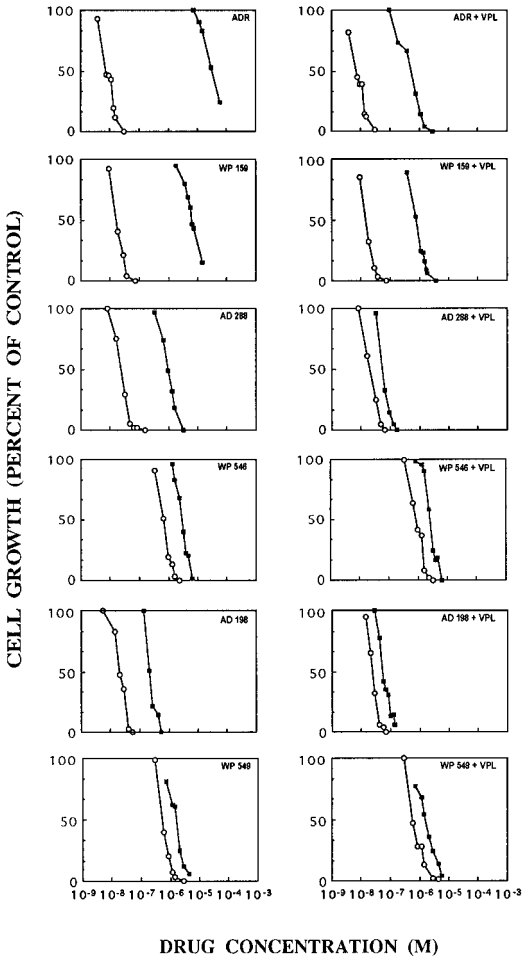

FIGURE 3: Comparison of growth inhibition of anthracycline analogs (left panel) and effects on anthracycline resistance by cotreatment with 10 μg/mL verapamil (right panel) in MDR− (○---○) and MDR+ (■---■) cells treated continuously for 3 days. Results are presented in the order of increasing lipophilicities (from top to bottom) of each of the anthracycline analogs, with the least lipophilic on top. Note greater resistance ratios for positively-charged anthracyclines (ADR, AD288, and AD198) vs neutral counterparts (WP159, WP546, and WP549) and reduced resistance ratios as a function of increased lipophilicity.

Table 2

	ID_{50} (M)			
	FLC + Vpl	ARN 15 + Vpl	ratio[a]	R/R (%)[b]
ADR	6.7×10^{-9}	5.84×10^{-7}	87	98.1
WP159	1.38×10^{-8}	7.64×10^{-7}	55	86.6
AD288	2.0×10^{-8}	4.96×10^{-8}	2.5	92.4
WP546	8.2×10^{-7}	2.56×10^{-6}	3	25.0
AD198	2.38×10^{-8}	5.34×10^{-8}	2	81.8
WP549	5.3×10^{-7}	1.53×10^{-6}	3	0

[a] ID_{50} ARN15 + Vpl/ID_{50} FLC + Vpl. [b] Ratio/resistance index (from Table 1).

inhibits the binding of the Vpl photoaffinity analog to P-gp (Figure 4). Although WP159 shows some interference with the Vpl analog it is significantly less than that of ADR at 250 μM. The other analogs, i.e., AD288, WP159, AD198, and AD549 show marked inhibition of Vpl analog binding to P-gp at both 50 and 250 μM. Thus, these results demonstrate that the anthracycline analogs with reduced resistance indices, i.e., AD198, WP546, AD288, and WP549, as well as ADR, with a high resistance index, interfere with Vpl binding to P-gp.

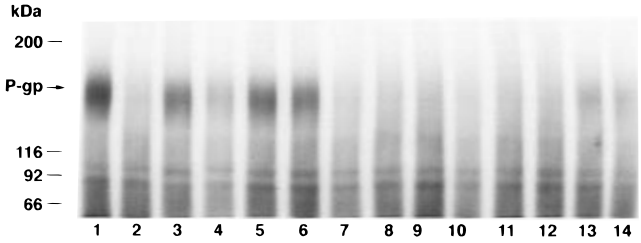

FIGURE 4: Autoradiogram of *N*-(*p*-azido-3-[^{125}I]salicil)aminomethyl verapamil, ([^{125}I]NAS-VP) photoaffinity labeled P-glycoprotein in ARN 15 cells. Photoaffinity labeling of ARN 15 cells in the absence (lane 1) or presence (lane 2) of 50 μM Vpl, 50 and 250 μM of ADR (lanes 3 and 4), WP 159 (lanes 5 and 6), AD 288 (lanes 7 and 8), WP 546 (lanes 9 and 10), AD 198 (lanes 11 and 12), and WP 549 (lanes 13 and 14), respectively. Photoaffinity analog of verapamil, [^{125}I]NAS-VP was synthesized as previously descibed (Safa, et al., 1988).

DISCUSSION

Since the discovery of clinical antitumor activity of ADR and daunorubicin, a large number of anthracyclines have been synthesized and studied (Arcamone, 1981; Lown, 1988; Priebe, 1995). *In vitro* studies have sporadically included the intracellular localization of some of these compounds (Lothstein, et al., 1993, 1995), however, little information is available on the structural requirements of anthracyclines for their differential localization in cells. Here, using a selected series of anthracycline analogs, we report a correlation between increasing lipophilicities, as measured by partition coefficients, and a change from predominantly nuclear to mitochondrial/cytoplasmic anthracycline localization. As previously shown with some of these compounds, their increased lipophilicities are associated with decreased affinities to bind native DNA (Israel et al., 1988). Although it has been assumed that anthracycline binding to DNA requires a free protonatable amino group on the glycosidic portion of the molecule (Arcamone, 1981), it has recently been shown that WP159, which contains an OH in the place of the NH_2 group, nevertheless binds native DNA (Priebe et al., 1995). Thus, our observations that WP 159 localizes in the nucleus can be explained, at least in part, by its DNA binding properties. Moreover, the nuclear localization of WP159 seems to correlate with recognition by MDR+ cells whereas the compounds which localize more in the cytoplasm are able to overcome MDR.

This latter point is well-illustrated with the findings that increasing lipophilicity of the anthracyclines studied here coincides with decreasing resistance indices, as shown in Table 1 and Figure 3 (left panel). The exception appears to be the 10-fold resistance observed with AD198, which is greater than the 4-fold MDR resistance to the theoretically less lipophilic neutral analog WP546. This result may be explained by the previously reported observation that AD198 converts into AD288 upon entering the cell due to esterase activity which cleaves the valerate portion of AD 198 (Lothstein et al., 1992).

On the basis of previous reports in which part of the resistance to anthracyclines in MDR cells has been shown to be due to alterations in the levels of topoisomerase II enzyme (Beck, 1990), our results here which show that cytoplasmic localizing anthracyclines overcome almost all of the high level of resistance found in these cell lines may be due to their intracellular localization and non-nuclear targets. Thus, an agent which does not target nuclear DNA

would not be susceptible to mechanisms of resistance that involve nuclear targets such as topoisomerase II. This idea is supported by previous work with the mitochondrial targeting agent Rho 123 which when administered to ARN 15 cells in conjunction with Vpl completely overcame the high level MDR found in this cell line as compared to cells cotreated with ADR and Vpl which continued to show a significant amount of resistance (Lampidis et al., 1985).

When compared to their neutral analogs, resistance indices of the protonatable analogs are much greater and are better modulated by Vpl (Table 2 and Figure 3, right panel). Fluorescence localization studies carried out on cardiac muscle and cardiac nonmuscle cells, which are intrinsically MDR− and MDR+, respectively, also showed that the positively-charged analogs were better distinguished by the MDR process than the neutral analogs. Both results correlate well with previous data in which we showed that zwitterionic rhodamine analogs were not differentiated by MDR+ cells while their positively-charged analogs were (Lampidis et al. 1989). However, here we also find that WP159 which does not carry a positive charge is clearly recognized by MDR+ cells. We interpret these data to mean that the positive charge of anthracyclines is not a necessary requirement for MDR recognition but that the presence of a basic nitrogen which can be protonated facilitates the processing of these compounds by the MDR efflux system.

Mechanisms to support this interpretation would include either (1) the electrostatic positive charge better positions the recognizable site of the anthracycline to a counter negative P-gp site and/or (2) positive charge allows for greater intracellular enrichment due to intracellular binding or trapping and thus, overall, increases drug internal accessibility to the P-gp export pump. Data previously reported by Priebe et al. (1995) and confirmed here in our MDR+ cell type suggests that the binding of WP159 to P-gp by photoaffinity analysis is approximately 10-fold less than that of ADR. Since these experiments were performed in live cells, the contribution of the electronegative charge of the membrane potential as well as negative electrostatic charges on the cell surface to attract positively-charged compounds must be considered. Even though it is generally accepted that the positive species of anthracyclines such as ADR do not traverse the plasma membrane due to their localized charge, nevertheless, charge may enhance molecule proximity to membranes for the neutral species to get in. Once the neutral form of a protonatable anthracycline enters the cell and becomes protonated due to differences in internal pH, the positively-charged species may (1) bind not only to DNA but to other negatively-charged cell components and/or (2) become ion-trapped within the cell. These mechanisms therefore would be expected to favor enhanced intracellular concentration of protonatable anthracyclines as compared to nonprotonatable analogs even though the charged species do not traverse the plasma membrane.

Additionally, the data presented here show that as lipophilicity is increased to a high enough value, i.e., log $p > 1.99$, MDR is reduced and almost abolished. This agrees with previous studies with other lipophilic cationic compounds in which we found that as lipophilicity increases, resistance indices decrease (Lampidis et al., 1989). These results as well as the data presented here could be explained on the basis that highly lipophilic anthracyclines, and other organic cations can override the efficiency of the pump via high speed of inward passive diffusion or that very lipophilic compounds stick to the glycoprotein and therefore do not get transported out.

Taking this latter explanation into account, highly lipophilic compounds could also theoretically act as modulators by attaching to the P-gp and thus interfering with substrate availability and efficiency of the pump. The latter point is suggested by our experiments with Rho 123 (not shown) which indicate much brighter florescence retention in mitochondria when resistant cells are cotreated with WP549 and Rho 123 than either drug alone. Since Rho 123 has been found not to have MDR modulatory activity, (Lampidis, T. J. unpublished results) the brighter fluorescence staining of mitochondria when these two drugs are used simultaneously most likely represents WP 159-induced increase in Rho 123 retention in these MDR+ cells.

Surprisingly, in a study of a series of lipophilic pyridinium congeners, resistance indices were found to increase rather than decrease as a function of increasing lipophilicity (Dellinger et al., 1992). The apparent discrepancy with these simple compounds and the anthracyclines could be explained by the observation that the pyridiniums do not act as self modulators. The speed of uptake therefore for the anthracyclines would thus appear to be a less important factor in the mechanism of circumventing MDR.

In addition, the results in which we find that the four highly lipophilic anthracycline analogs markedly inhibit the binding of the photoaffinity analog of verapamil to P-gp provide further evidence which suggests that these analogs act as modulators. In fact their lower resistance ratios may indeed be reflecting self-modulation leading to their increased intracellular accumulation. The presence of a pendant bulky group in these four anthracyclines coincides with previous results in which it was shown in a series of yohimbe and reserpine analogs that only those compounds with pendant bulky groups had MDR modulatory activity (Pearce et al., 1989). Moreover, drug transport in MDR+ cells and photoaffinity labeling of P-gp can be interfered with by detergents, as shown by Sehested et al. (1988) and Friche et al. (1993). Although our compounds do not appear to act as detergents, nevertheless, it remains a possibility that the inhibition of labeling by AD198, WP546, AD288, and WP549 could be through allosteric membrane perturbation.

Although absolute intracellular localization to the nucleus or cytoplasm probably does not occur with any of the anthracyclines tested, a general pattern which is supported by our studies is that, as a function of decreased affinity for binding DNA, anthracyclines shift in their primary localization from nuclear to mitochondrial to diffuse cytoplasmic. Since the localization shift appears to also correlate with increasing lipophilicities of the six compounds studied, it remains to be determined how each of these parameters interact to affect anthracycline intracellular localization.

DNA fluorescence quenching of anthracyclines is known to affect the apparent localization properties of these compounds. Since three of the compounds (WP546, AD198, WP549) that we find more localized in the cytoplasm and less localized in the nucleus are shown to bind DNA less avidly than ADR, or not at all, our results most likely reflect their true intracellular distributions. To further verify this, experiments were performed where nuclei of MDR− cells were isolated, treated with each of the drugs, subjected to extraction procedures, and measurements for drug levels were

made via HPLC. The results of these experiments showed that WP549, AD198 and WP546 concentrate in nuclei very little or not at all as compared to the amounts of ADR, WP159, and AD288. Thus, the results of our localization studies in intact cells which showed that ADR and WP 159 were found mostly in nuclei while AD288 showed nuclear and cytoplasmic staining correlate with our isolated nuclear assays. Moreover, the very little extracted amounts of W546, AD198, and WP549 from treated isolated nuclei support our localization results in intact cells in which we find very little nuclear staining. Overall, these results most likely reflect the intrinsic ability of each of these drugs to bind native DNA. The result that AD288 but not the other highly lipophilic anthracyclines shows high accumulation in isolated nuclei may also be explained by differences in DNA binding.

In summary, the results presented which most likely could be relevant to the clinical application of anthracyclines are as follows: (1) Highly lipophilic anthracyclines overcome extremely high levels of ADR resistance most of which is due to P-gp-mediated MDR. (2) Localization of these anthracyclines are predominantly in the cytoplasm and mitochondria, which may contribute to their circumvention of resistance mechanisms other than those due to P-gp MDR, i.e., topoisomerase II. (3) Self-modulation by highly lipophilic anthracyclines may be an important mechanism to overcome P-gp MDR.

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