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IMPROVED CELLULAR ACCUMULATION IS CHARACTERISTIC OF ANTHRACYCLINES WHICH RETAIN HIGH ACTIVITY IN MULTIDRUG RESISTANT CELL LINES, ALONE OR IN COMBINATION WITH VERAPAMIL OR CYCLOSPORIN A

HELEN M. COLEY, PETER R. TWENTYMAN and PAUL WORKMAN*
MRC Clinical Oncology and Radiotherapeutics Unit, Hills Road, Cambridge CB2 2QH, U.K.

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Abstract—We have examined the cellular accumulation of anthracycline compounds, alone or in conjunction with resistance modifiers, in an attempt to identify mechanisms by which multidrug resistance (MDR) can be circumvented. This was facilitated by using the EMT6 mouse mammary tumour cell line EMT6/P and its MDR subline EMT6/AR1.0 with 30-fold resistance to Adriamycin® (ADM), and the human small cell lung cancer line H69/P together with its MDR subline H69/LX4 with 100-fold resistance to ADM. Both MDR lines hyperexpress membrane P-170 glycoprotein. The accumulation of ADM was compared to that seen for the anthracycline analogues aclacinomycin A (ACL), Ro 31–1215 and 4′-deoxy-4′-iodo-Adriamycin® (iodo-ADM). These analogues were selected because of their high activity against MDR sublines, including H69/LX4 and EMT6/AR1.0. Both MDR cell lines exhibited a deficiency in ADM accumulation compared to the parent lines. Smaller differentials were seen using Ro 31–1215 or iodo-ADM. Both resistant sublines were able to accumulate ACL in identical amounts to their respective parental sublines. Improved drug accumulation is likely to contribute to the improved activity of the analogues against MDR cell lines. However, the relative accumulation defects in the resistant lines did not correlate exactly with the degree of resistance to a particular compound. Cyclosporin A (5 μg/ml) or verapamil (3.3 μg/ml) caused a preferential increase in uptake in both MDR sublines, with a small or negligible effect for the parental line. A smaller effect was observed with iodo-ADM and Ro 31-1215, and levels of ACL were unchanged in the MDR lines in the presence of either resistance modifier. These results indicate two mechanisms for circumventing drug resistance due to reduced drug accumulation. Structurally modified derivatives can partially or completely eliminate uptake differentials between parent and drug resistant cell lines. Any residual uptake can be eliminated using resistance modifiers. The two mechanisms may both operate via inhibition or circumvention of P-170 mediated efflux. The situation is complex, however, and this study indicates the possible involvement of additional resistance mechanisms.

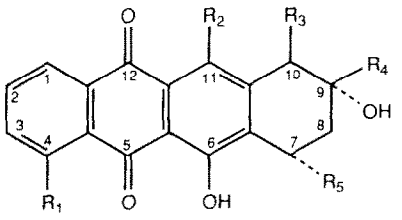
The precise mechanisms by which multidrug-resistant (MDR) cell lines achieve lower intracellular cytotoxic drug levels than their corresponding drug sensitive lines is poorly understood. Two main mechanisms have been proposed in an attempt to explain this: (i) reduced membrane permeability leading to decreased rate of entry of drug [1–3] and (ii) an accelerated rate of drug removal from the cell via an energy-dependent efflux mechanism [4–6]. Due to the common finding in MDR cell lines of a 170 kD membrane glycoprotein—known as P-glycoprotein—with structural homology to well-characterized membrane transport proteins [7, 8], a causal link between hyperexpression of this protein and reduced drug accumulation is suspected [9, 10]. It is envisaged that certain drugs may bind as substrates which in turn enables them to be effluxed by the P-glycoprotein molecule. This is supported by the demonstration of increased membrane binding of vincristine and related analogues in an MDR human KB carcinoma cell line [11] as well as the ability of resistance modifying agents to act as ligands for P-glycoprotein [12–14].

We have previously identified certain structurally modified anthracyclines capable of circumventing Adriamycin® (doxorubicin hydrochloride) (ADM)† resistance in MDR cell lines *in vitro* [15]. These include 4′-deoxy-4′-iodo-ADM (iodo-ADM) where the modification is to the amino sugar unit, the analogue Ro 31-1215 where the important substitution is an alkyl group at the 9-position off the A-ring, and aclacinomycin A (ACL) which possess both the 9-alkyl substitution and a markedly altered sugar moiety (Fig. 1). Such anthracyclines which retain high activity in MDR cell lines have clear potential for clinical evaluation. To further investigate the mechanism by which these analogues retain high activity we have investigated their cellular pharmacokinetics, in comparison with those for ADM, in parent and MDR cell lines derived from EMT6 mouse mammary tumour and H69 human small cell lung cancer lines. Resistance was produced by exposure to ADM *in vitro*, and both lines show hyperexpression of P-170 glycoprotein [16].

The resistance modifiers verapamil (VRP), a calcium channel blocker, and cyclosporin A (CYA), a calmodulin antagonist, have been shown previously

*To whom correspondence should be addressed.

† Adriamycin is a registered trademark of Farmitalia Carlo Erba.



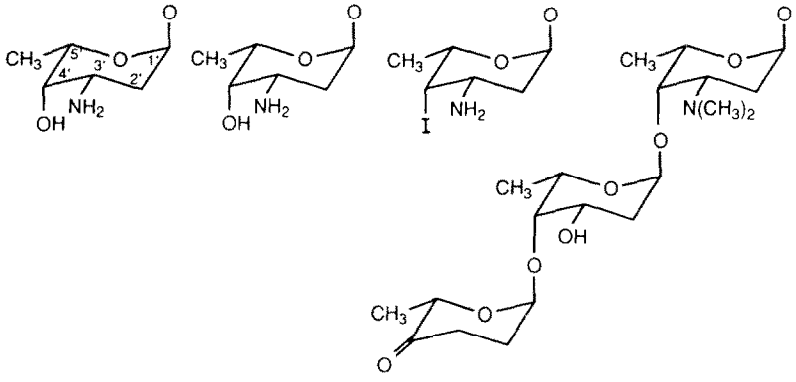
	Adriamycin	Ro 31-1215	4'-Deoxy-4'-iodo-adriamycin	Aclacinomycin A
R_1	OCH_3	H	OCH_3	OH
R_2	OH	OH	OH	H
R_3	H	H	H	$COOCH_3$
R_4	$COCH_2OH$	CH_3	$COCH_2OH$	CH_2CH_3
R_5				

Fig. 1. Structures of anthracyclines.

to exhibit selective enhancement of anthracycline response in MDR cell lines as compared to the corresponding drug sensitive cell lines [15–21]. Indeed, the combination of those anthracyclines which show preferential activity over ADM together with resistance modifying agents *in vitro* has been shown to completely circumvent the MDR phenotype in some instances [15]. Various resistance modifiers have been shown to affect intracellular drug levels by inhibiting drug efflux [17–21]. We have therefore also investigated the effects of VRP and CYA on the accumulation of iodo-ADM, ACL and Ro 31-1215 in the present study.

The results indicate that Ro 31-1215, ACL and iodo-ADM exhibit improved intracellular accumulation in MDR cells lines compared to that seen for ADM, thus contributing to their enhanced activity. In addition, the effect of resistance modifiers on the intracellular accumulation of these anthracyclines is less than for ADM, as is the case for the modification of cytotoxicity in the same cell lines. The goal of these and future studies is to fully elucidate the relationship between the modified expression of specific resistance-associated proteins and the altered cellular handling of anthracyclines in MDR cell lines.

MATERIALS AND METHODS

Cell lines and culture conditions. The parent NCI-H69 human small cell lung cancer line (designated H69/P) and its *in vitro* derived ADM-resistant variant (H69/LX4) [22] were grown as floating aggregates in RPMI 1640 medium (Gibco Biocult, Paisley, U.K.) with 10% foetal calf serum (Seralab, Crawley Down, U.K.), penicillin and streptomycin (at concentrations of 100 units/ml and 100 μg/ml, respectively). Stock cultures were maintained in 15 ml of medium in 75 cm^2 tissue culture flasks at 37° in an atmosphere of 92% air, 8% CO_2. The H69/LX4 variant was maintained in 0.4 μg/ml ADM but the drug was removed at least 2 days before use in experiments. Cells were harvested from cultures in the exponential phase of growth.

The parent mouse mammary tumour cell line EMT6/Ca/VJAC (hereafter referred to as EMT6/P) was maintained as a monolayer in Eagle's minimal essential medium with 20% new born calf serum (Gibco Biocult) and antibiotics in 75 cm^2 flasks under similar conditions to those used for H69. The resistant variant EMT6/AR1.0 [23] was routinely maintained in 1.0 μg/ml ADM until 2 days before

experimental use. Again, cells were harvested from the exponential growth phase.

H69 cultures for use in experiments were reduced to a suspension containing small groups of cells by pipetting. Cell counts were carried out by taking an aliquot of the suspension and incubating it with 0.4% trypsin and 0.02% versene in phosphate buffered saline (PBS) for 15 min at 37°. The single cell suspension was counted manually using a haemocytometer counting chamber. Based on this count, the mechanically disaggregated suspension was diluted as appropriate.

The EMT6 monolayers were subjected to two rinses with 0.1% trypsin in PBS followed by a 15 min incubation at 37°. A single cell suspension was obtained by resuspension of cells in full Eagle's medium with mechanical disaggregation, and counted and diluted as for the H69 cell lines.

Drugs and chemicals. We are grateful for the gifts of the following compounds: Ro 31-1215 from Roche Products Ltd. (Welwyn Garden City, U.K.); 4'-deoxy-4'-iodo-ADM (iodo-ADM) from Farmitalia Carlo Erba (Milan, Italy); aclacinomycin A (ACL) from Lundbeck Ltd. (Luton, U.K.); and cyclosporin A (CYA) from Sandoz Ltd. (Basel, Switzerland). ADM was obtained from Sigma Chemical Co., (Poole, U.K.) and verapamil (VRP) was supplied by Abbot Laboratories (Queenborough, U.K.).

All cytotoxic drugs except ACL were dissolved in distilled water at 500 μg/ml, filtered via a Millipore membrane (pore size 0.2 μm) and stored in aliquots at −20°. ACL was dissolved in 0.1% propylene glycol. Drugs were thawed and diluted in distilled water immediately before use. VRP was obtained as a 250 μg/ml aqueous solution and diluted in PBS. CYA was initially dissolved in absolute ethanol and diluted in PBS prior to use. The final concentration of ethanol (0.1% v/v) and propylene glycol (0.002% v/v) was shown not to affect drug sensitivity or cell growth.

Drug accumulation. The method used to determine the anthracycline content per cell was essentially that of Schwartz [24]. Single cell suspensions were prepared as described above to give a concentration of 1×10^6 cells in 5 ml aliquots of appropriate complete medium (at pH 7.4) in duplicate 10 ml polypropylene centrifuge tubes. Cell suspensions were brought to 37° and drug solutions were added in 100 μl volume. Final concentrations were 1 or 10 μg/ml, according to the intrinsic fluorescence of the individual compounds. This corresponded to 5 μg/10^6 cells for Ro 31-1215 and iodo-ADM, and 50 μg/10^6 cells for ACL and ADM. During the incubation period, tubes were agitated at 10 min intervals.

At the appropriate time points, the tubes were centrifuged rapidly at 4° (300 *g* for 2 min) and the cells were washed twice in ice-cold Hank's balanced salt solution (HBSS), pH 7.4. A volume of 0.2 ml of ice-cold sodium lauryl sulphate solution (0.1%) was then added and the tubes were mixed using a vortex mixer. A volume of 0.2 ml of ice-cold silver nitrate (33% w/v) was then added and the tubes were shaken for 10 min at 4°. At the end of this time, 4 ml of iso-amyl alcohol was added followed by a further 10 min shaking period and centrifugation for 5 min at 200 *g*. The alcohol layer was then transferred to a 5 ml boro-silicate glass tube and fluorescence was measured using a Perkin Elmer MPF4 spectrofluorimeter. The excitation (Ex) and emission (Em) wavelengths used to measure the individual anthracyclines were 490 nm (Ex) and 595 nm (Em) for ADM, 450 nm (Ex) and 570 nm (Em) for ACL, 480 nm (Ex) and 590 nm (Em) for iodo-ADM and 485 nm (Ex) and 565 nm (Em) for Ro 31-1215. Standards were prepared by adding appropriate amounts of anthracycline to tubes containing untreated cells followed by the immediate addition of sodium lauryl sulphate and silver nitrate. The fluorescence due to anthracycline content was shown to be linear with concentration and very stable over a 48 hr period in the dark with no deterioration during the period of analysis.

Effect of resistance modifiers. The resistance modifiers were added in 100 μl volumes to give a final concentration of 3.3 μg/ml for VRP and 5 μg/ml for CYA in a total of 5 ml of cell suspension. These concentrations were previously selected by us to represent two to three times the clinically achieveable peak level, and to cause a 20-fold increase in ADM-sensitization in the H69/LX4 line [15]. Control tubes were set up simultaneously containing 100 μl of the appropriate solvent. Aliquots of anthracycline were then added immediately at time zero.

All experimental data points are the mean of at least two separate sets of duplicate determinations. The inter-assay coefficient of variation was about 5% for both Ro 31–1215 and iodo-ADM, 5–16% for ADM and 7–15% for ACL.

RESULTS

Accumulation of anthracycline analogues

The effectiveness of Ro 31-1215, ACL and iodo-ADM in circumventing MDR in the two resistant sublines has been reported previously [15]. Results were expressed as resistance factors (RF = ID_{50} for resistant line/ID_{50} for parental line). In H69 these were 150 for ADM, 12.4 for Ro 31-1215, 5.8 for ACL and 18.9 for iodo-ADM. In EMT6 ADM gave an RF value of 33.9 compared to 8.1 for Ro 31-1215, 4.7 for ACL and 4.4 for iodo-ADM.

Figures 2 and 3 show the typical drug accumulation patterns obtained with ADM and the anthracycline analogues in H69/P, EMT6/P and their respective MDR cell lines. Both resistant counterparts are defective in accumulation of ADM, particularly EMT6/AR1.0. For example, at 2 hours the intracellular drug level was 16-fold lower in the case of EMT6/AR1.0 and 2.5-fold lower for H69/LX4, as compared to the respective parent line.

In contrast to those for ADM, the drug accumulation profiles for the three anthracycline analogues showed a very rapid rise within the first 10 min for both parent and resistant lines alike. This was followed by a slower uptake phase (Figs 2 and 3).

It can be seen that the accumulation of Ro 31-1215 in EMT6 showed a smaller differential between parent and resistant lines compared to that seen for ADM, with a difference of only 2-fold at 2 hours. A similar pattern was obtained with iodo-ADM where the differential was 1.5-fold at 2 hours. Interestingly,

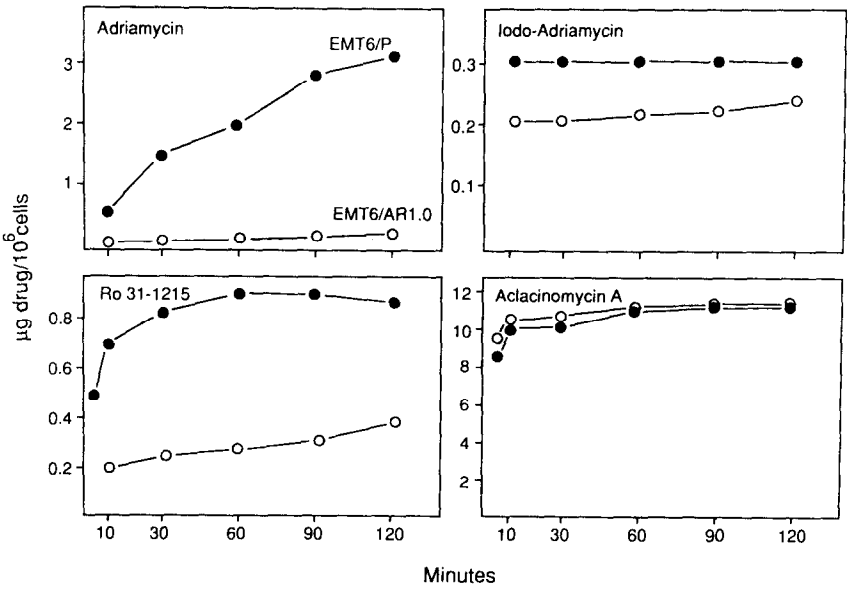


Fig. 2. Accumulation of anthracyclines in the EMT6/P (●) and EMT6/AR1.0 (○) cell lines, measured at 37°. Cells were exposed to Adriamycin® (10 μg/ml), iodo-adriamycin (1 μg/ml), Ro 31-1215 (1 μg/ml) or aclacinomycin A (10 μg/ml). Data points are the mean of duplicate samples from each of two independent experiments.

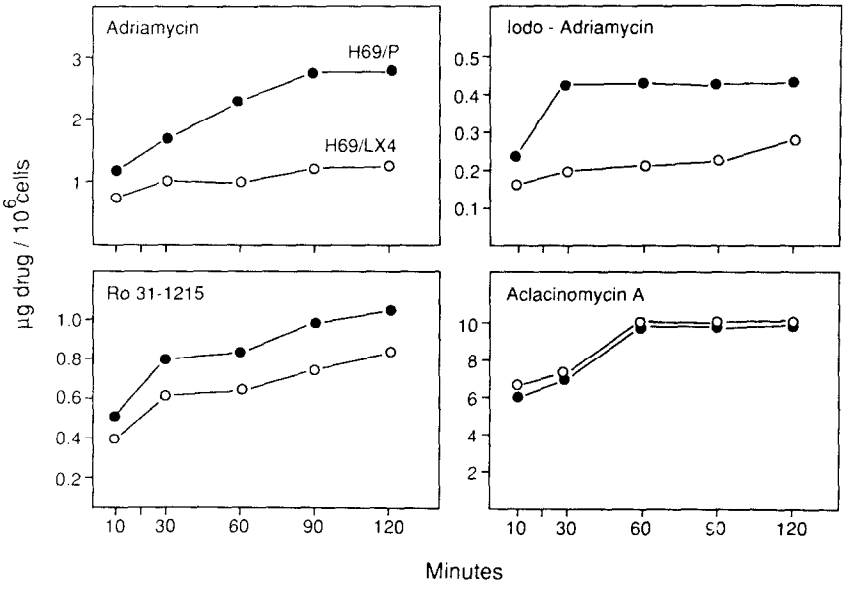


Fig. 3. Accumulation of anthracyclines in the H69/P (●) and H69/LX4 (○) cell lines. Other details are as for Fig. 2.

EMT6/AR1.0 was as effective as the EMT6/P line in the accumulation of ACL.

For the H69 cell lines we again saw smaller differentials in drug accumulation between parent and resistant lines for both iodo-ADM and Ro 31-1215, as compared to those for ADM (Fig. 3). Likewise, the ACL accumulation was similar in the resistant H69/LX4 line to that in the H69/P line.

Thus it is clear that Ro 31-1215, iodo-ADM and ACL all showed improved accumulation in both the MDR cell lines examined, as compared with ADM.

Anthracycline accumulation with resistance modifiers

The effects of the resistance modifiers VRP and CYA on the accumulation of the various anthracyclines was determined in both the EMT6 and H69 cell lines. The results are summarized in Tables 1 and 2 as the 1 hour and 4 hour values respectively. The steady state drug levels are reached between 2 and 4 hours (unpublished data) consistent with the findings of others [25, 26]. Some selected examples of drug accumulation are illustrated in Figs 4 and 5.

Figure 4 demonstrates the marked increase in

Table 1. One hour drug accumulation values in the presence or absence of resistance modifiers

Cell Line	Expt No.		Cellular accumulation ($\mu g/10^6$ cell)			
			ADM	Ro 31-1215	Iodo-ADM	ACL
EMT6/P	1	Control	2.0	0.9	0.5	6.4
		VRP	2.0 (1.0)	1.0 (1.1)	0.5 (1.0)	6.4 (1.0)
	2	Control	2.0	0.7	0.4	10.5
		CYA	2.2 (1.1)	0.7 (1.0)	0.4 (1.0)	10.5 (1.0)
EMT6/AR1.0	1	Control	0.1	0.3	0.2	6.6
		VRP	0.7 (5.6)	0.8 (2.7)	0.4 (2.0)	6.6 (1.0)
	2	Control	0.4	0.3	0.2	11.5
		CYA	1.7 (4.3)	0.7 (2.3)	0.4 (2.0)	11.5 (1.0)
H69/P	3	Control	0.8	0.4	0.9	10.0
		VRP	1.0 (1.2)	0.4 (1.0)	1.0 (1.1)	10.0 (1.0)
	4	Control	1.0	0.8	0.8	8.3
		CYA	1.1 (1.1)	0.9 (1.1)	0.8 (1.0)	9.0 (1.1)
H69/LX4	3	Control	0.5	0.4	0.7	10.5
		VRP	1.2 (2.2)	0.5 (1.2)	1.2 (1.7)	10.5 (1.0)
	4	Control	0.5	0.6	0.6	9.5
		CYA	1.2 (2.4)	1.0 (1.7)	0.8 (1.3)	9.5 (1.0)

Values are the mean of duplicate determinations. The number in parenthesis is the increase in cellular amount of drug as compared to the control (without modifier).

Table 2. Four hour accumulation values in the presence or absence of resistance modifiers

Cell Line	Expt No.		Cellular accumulation (μg drug/10^6 cells)			
			ADM	Ro 31-1215	Iodo-ADM	ACL
EMT6/P	5	Control	3.6	1.4	0.5	11.5
		VRP	4.0 (1.1)	1.5 (1.1)	0.5 (1.0)	11.5 (1.0)
	6	Control	3.5	1.1	0.5	11.5
		CYA	3.6 (1.0)	1.2 (1.1)	0.6 (1.1)	12.3 (1.1)
EMT6/AR1.0	5	Control	0.6	0.7	0.3	12.0
		VRP	3.2 (5.3)	1.3 (1.8)	0.5 (1.7)	12.1 (1.0)
	6	Control	0.4	0.5	0.3	12.0
		CYA	3.2 (8.0)	1.0 (2.0)	0.5 (1.7)	12.3 (1.0)
H69/P	7	Control	2.8	0.9	1.2	11.6
		VRP	2.9 (1.0)	1.1 (1.1)	1.2 (1.0)	11.5 (1.0)
	8	Control	2.8	0.9	1.4	11.6
		CYA	2.8 (1.0)	0.9 (1.0)	1.4 (1.0)	11.6 (1.0)
H69/LX4	7	Control	1.1	0.5	0.9	11.8
		VRP	2.5 (2.3)	0.7 (1.4)	1.2 (1.0)	11.8 (1.0)
	8	Control	1.1	0.7	0.9	11.8
		CYA	2.6 (2.4)	1.1 (1.6)	1.1 (1.0)	11.8 (1.0)

Footnotes as for Table 1.

cellular ADM content in the EMT6/AR1.0 line in the presence of either VRP or CYA. This contrasts markedly with the minimal effects seen for the corresponding parent EMT6/P line. Data in Tables 1 and 2 confirm this preferential effect to occur also in the H69/LX4 line as opposed to the H69/P line, measured at both 1 and 4 hours.

Little change in accumulation of Ro 31-1215 in the EMT6/P line occurred following VRP treatment. Substantial increments in drug levels were seen in the EMT6/AR1.0 line at both 1 and 4 hours, but they were less than those obtained for ADM. In the same experiments CYA had a negligible effect on Ro 31-1215 accumulation in the H69/P and EMT6/P lines, whilst both resistant counterparts showed substantial increases. Similar patterns were seen for iodo-ADM in combination with either VRP or CYA.

The accumulation of ACL was markedly different from that seen for the other compounds studied. Resistance modifiers had little effect on ACL accumulation in any of the cell lines, drug resistant or drug sensitive (Tables 1 and 2).

Overall, these data show VRP at 3.3 μg/ml and CYA at 5 μg/ml to exert similar quantitative and qualitative effects on drug accumulation. Using either resistance modifier, the reduced drug accumulation seen in the drug resistant cell lines

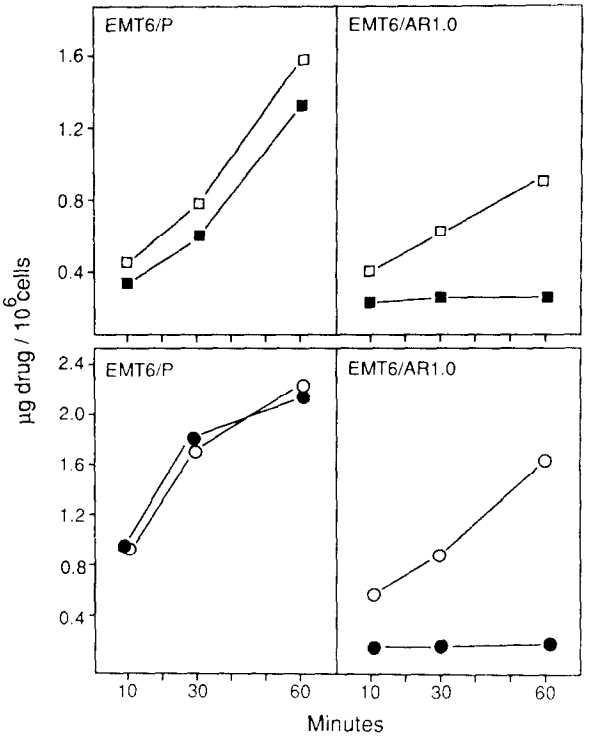


Fig. 4. Accumulation of Adriamycin® (10 µg/ml) in EMT6/P (left hand panels) or EMT6/AR1.0 (right hand panels) in the presence (□) or absence (■) of verapamil (3.3 µg/ml), or in the presence (○) or absence (●) of cyclosporin A (5 µg/ml) at 37°. Data points are the mean values of duplicate samples from each of two independent experiments.

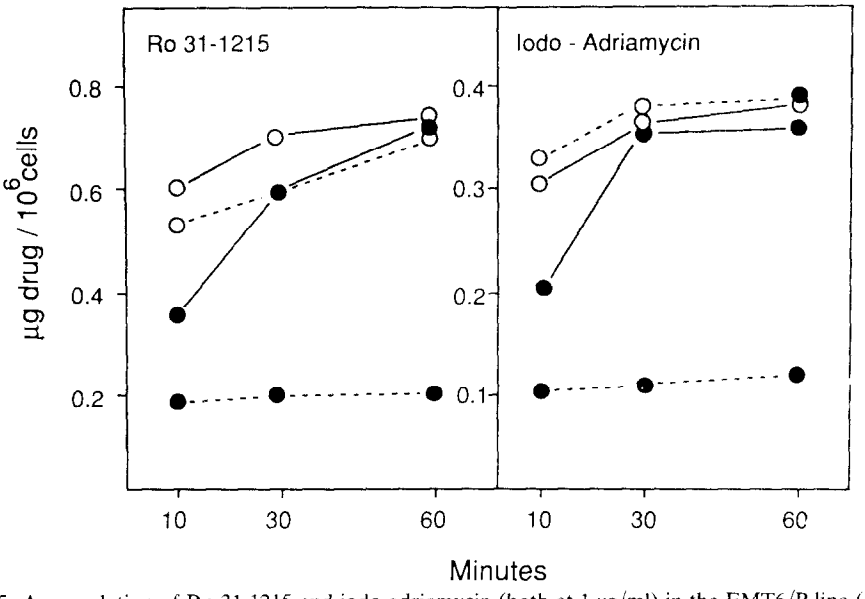


Fig. 5. Accumulation of Ro 31-1215 and iodo-adriamycin (both at 1 µg/ml) in the EMT6/P line (——) and the EMT6/AR1.0 line (---), in the presence of (○) or absence (●) of verapamil (3.3 µg/ml) at 37°. Data points are the mean values of duplicate samples from each of two independent experiments.

was increased to levels approaching those seen in the respective parent drug sensitive lines. In the case of ACL no further modification was observed with either VRP or CYA. The greatest modification was seen for ADM, with the changes for Ro 31-1215 and iodo-ADM intermediate between ADM and ACL.

DISCUSSION

We describe cellular drug accumulation studies using anthracyclines to which MDR cell lines exhibit minimal cross-resistance [15, 23, 27]. Two cultured cell line pairs were used: (1) the drug sensitive H69 small cell lung cancer line H69/P together with its

ADM-resistant counterpart H69/LX4 which shows approximately 100-fold resistance to ADM; and (2) the drug sensitive EMT6 mouse mammary tumour cell line and its ADM resistant subline EMT6/AR1.0 with a 30-fold resistance to ADM. Both H69/LX4 and EMT6/AR1.0 exhibit a spectrum of cross-resistance including resistance to vincristine, colchicine and etoposide, consistent with the MDR phenotype, and both cell lines express increased levels of P-glycoprotein mRNA and protein product [16].

The degree of ADM resistance (obtained by comparing ID_{50} values) did not correlate exactly with the extent of defective drug accumulation. In the murine EMT6/AR1.0 line, ADM levels were reduced by 16-fold compared to those in the parent at 2 hours, whereas the more resistant H69/LX4 line showed only a 3-fold reduction. Since both cell line pairs exhibited similar dose–response curves, as measured in the MTT assay [15, 28], this discrepancy would also hold true if a different level of response was employed (e.g. ID_{90} as opposed to ID_{50}). It is therefore possible that the ADM-resistance in H69/LX4 and TMT6/AR1.0 is due to factors in addition to intracellular drug accumulation. Altered drug detoxification and/or the subcellular distribution of ADM may be important factors and will form tht focus of future studies.

The anthracycline compounds under review have modifications to the amino sugar group and/or possess an alkyl group at position 9 of the A-ring. They are more lipophilic than the parent compound, as demonstrated by octanol:water partition coefficient [23] and Folch's partitioning ratio [29]. It is clear however that the degree of lipophilicity is not solely responsible for chemosensitivity in MDR cell lines [3, 23].

The three analogues studied, Ro 31-1215, iodo-ADM and ACL, all exhibited a smaller differential in accumulation between the parent and corresponding MDR cell line compared to ADM. This was particularly true for ACL where the drug accumulation profiles were almost identical. It can be seen, however, that the degree of defective drug accumulation does not correspond directly to the extent of resistance to the different drugs. For example, in the EMT6 cell lines ACL and iodo-ADM show markedly different accumulation in spite of very similar degrees of resistance to these compounds. Nevertheless, the present studies show that modification of drug accumulation by molecular substitution in the anthracycline structure and/or the use of resistance modifiers can contribute substantially to the circumvention of the MDR phenotype.

In addition to the reduced differential in the ability of the parent and resistant lines to accumulate the anthracycline analogues, as compared to that seen with ADM, both CYA and VRP were able to further enhance cellular levels of Ro 31-1215 and iodo-ADM in the resistant line. The effect of the resistance modifiers on the cellular pharmacokinetics of these anthracyclines was somewhat lower than with ADM, but the final result in terms of overcoming MDR-associated reduced drug accumulation was the same. Drug resistant cell lines were able to accumulate similar drug levels to their parental drug sensitive counterparts, in which only a marginal effect was seen. In contrast, CYA-mediated enhancement of *in vivo* efficacy and cellular accumulation in a daunomycin (DNR)-sensitive Ehrlich ascites line has been reported [30]. In addition, the EMT6/P cell line has been shown to be more sensitive to VRP modification of ADM-sensitivity than the corresponding drug resistant line EMT6/AR1.0 [15, 16]. It would appear this cannot be explained in terms of changes in drug accumulation, as the VRP-induced ADM accumulation of ADM is substantial in the EMT6/AR1.0 line, and minimal in the EMT6/P line. The role of resistance modifiers in the circumvention of MDR is clearly complex.

ACL exhibited similar accumulation in the EMT6/AR1.0 and H69/LX4 drug-resistant cells compared to the drug-sensitive EMT6/P and H69/P cells, and no further effect was seen with the resistance modifiers. These results for the accumulation of ACL alone are in agreement with those of Tapeiro [31] and Seeber [25] using a DNR-resistant Ehrlich ascites tumour line which was not cross-resistant with ACL. The inability of VRP and CYA to enhance further uptake of ACL into the MDR cell lines suggests that the structural modifications of the anthracyclines on the one hand and the resistance modifiers on the other may exert their beneficial effects on accumulation and sensitivity via a shared mechanism.

These interesting results show that drug resistance due to reduced drug accumulation can be modified in two ways. Use of structurally modified derivatives can partially or completely eliminate the uptake differential between parent and resistant cells. Any residual accumulation defect can be corrected using resistance modifiers.

There is a common, though not universal, correlation between multidrug resistance to anthracyclines such as ADM with (1) defective accumulation and (2) up-regulation of P-170 glycoprotein [32–36]. This suggests that a major mechanism involves enhanced drug efflux, via the P-170 molecule, which has significant structural homology with ATP-driven transmembrane pump proteins [7, 8]. We propose that certain anthracyclines such as the 9-alkyl and amino sugar modified derivatives represent less efficient substrates for this putative efflux pump compared to ADM. It may be that ACL is not effluxed to any significant extent by this mechanism. According to the pump model the resistance modifiers operate by inhibiting anthracycline efflux [19] and it would therefore be predicted that they would have no effect on ACL accumulation. Effects of resistance modifiers on iodo-ADM and Ro 31-1215 would be intermediate between ACL and ADM. These predictions are supported by the data presented here.

To further test this model we are currently working to establish the precise relationship between P-170 hyperexpression and cellular pharmacokinetics, particularly the efflux of ADM and the anthracycline analogues shown to be effective against MDR cell lines. However, whilst reduced drug accumulation is a common feature of the MDR phenotype in most cell lines, it is becoming apparent that the two phenomenon may not be directly linked. No enhanced ADM efflux was seen in an MDR P388

leukaemia line showing hyperexpression of P-170 [32]. Conversely we have seen reduced accumulation and enhanced efflux of ADM in an atypical MDR cell line which does not hyperexpress membrane P-170 (unpublished data). MDR is clearly a complex process involving multiple mechanisms [34–37] and numerous membrane and cytosolic proteins [16, 35, 38–40]. As far as the most common form of *in vitro* derived MDR is concerned, however, the use of structurally modified anthracyclines together with resistance modifiers to improve cellular drug accumulation appears a particularly promising therapeutic approach.

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