Resistance to Anthrapyrazoles and Anthracyclines in Multidrug-resistant P388 Murine Leukemia Cells: Reversal by Calcium Blockers and Calmodulin Antagonists

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ABSTRACT

A series of anthrapyrazoles was examined for their cytotoxic effect on P388 cells resistant (P388R) to anthracyclines, N-[4-(9-acridinylamino)-3-methoxyphenyl]methanesulfonamide, trimetrexate, and vinblastine. The degree of resistance of P388R cells to Adriamycin (ADR) and daunomycin was 50-fold and 38-fold, respectively, when compared to the parent cell line (P388S). The Adriamycin-resistant cells were highly cross-resistant to some anthrapyrazoles, but the degree of cross-resistance was not uniform and was less than 3-fold for one member of the series. The lipophilicity of these compounds appeared to correlate to some extent with the level of resistance. The calcium channel blockers verapamil (VER) and diltiazem and the calmodulin antagonist trifluoperazine potentiated the cytotoxicity of the anthrapyrazoles and ADR in P388R. This potentiating effect was concentration dependent with VER being the most efficacious. VER increased ADR cytotoxicity by greater than 10-fold and CI-937 by almost 40-fold. However, VER, diltiazem, and trifluoperazine had no effect on ADR or anthrapyrazole activity in P388S cells. The antiarrhythmic drug, quinidine, and the detergent, Tween 80, also potentiated ADR activity in P388R cells to the same extent as VER. Both the net accumulation and efflux of [3H]daunomycin were altered in P388R cells by nontoxic concentrations of Tween 80 in a fashion virtually identical to that demonstrated for VER. These data suggest that agents which potentiate drug cytotoxicity in P388R cells may do so by their interaction with the lipid domain of the plasma membrane. In addition, these results demonstrate that some members of the new series of DNA binding drugs, the anthrapyrazoles, may be active against anthracycline-resistant tumors and that, where cross-resistance to them occurs, it can be partially reversed by agents such as VER.

INTRODUCTION

The development of resistance by tumor cells to certain natural product antitumor drugs is often associated with cross-resistance to other unrelated antineoplastic agents (1–8). For example, tumors that acquire resistance to ADR2 are also frequently resistant to other DNA binding drugs (1, 2) as well as plant alkaloids (3, 4) and other structurally dissimilar compounds (5–7). This multidrug resistance appears to be associated with altered membrane permeability, resulting in a decreased drug uptake and/or efflux (1, 3–5, 8–11), although other lesions have been described (12,13). Multidrug resistance of tumors represents one of the critical limitations in the treatment of cancer patients, and its circumvention is a major challenge in cancer chemotherapy. The recent reports describing the ability of calcium channel blockers (3, 14–17) and calmodulin antagonists (15–18) to potentiate drug activity in resistant cell lines may represent one avenue for overcoming drug resistance.

The anthrapyrazoles are a new class of DNA binding anticancer drugs (19) which will soon enter clinical trials. These drugs have been demonstrated to have a high level of broad spectrum activity against a large number of in vivo animal tumor models (20). In the present study, we evaluated several of these new drugs against an ADR-resistant P388 leukemia cell line and determined the ability of calcium and calmodulin antagonists to potentiate their activity.

MATERIALS AND METHODS

Cell Lines. P388 murine leukemia cells (P388S) and an ADR-resistant subline (P388R) obtained from the Southern Research Institute (Birmingham, AL) were cultured from ascites fluids of tumor-bearing DBA/2 mice and maintained in Fischer's medium supplemented with 10% horse serum, 10 μM 2-mercaptoethanol, and gentamycin (50 μg/ml).

The effect of anticancer agents alone or in combination with calcium or calmodulin antagonists on the growth of both cell lines was determined as previously described (21). Cells were counted with a Model Zr Coulter Counter (Coulter Electronics, Inc., Hialeah, FL). Viability was determined by trypan blue exclusion.

Chemicals. [3H]Daunomycin, specific activity, 5 Ci/mmol, was purchased from New England Nuclear, Boston, MA. Unlabeled daunomycin was purchased from Sigma Chemical Co., St. Louis, MO. The anthrapyrazoles were obtained from the Warner-Lambert Co. Fischer's medium and horse serum were purchased from K. C. Biological, Inc., Lenexa, KS. Adriamycin, quinidine sulfate, verapamil, diltiazem, trifluoperazine, W-7, ethidium bromide, and Tween 80 were purchased from Sigma Chemical Co., St. Louis, MO.

Transport Studies. The uptake of [3H]DAU (0.25 nM [3H]daunomycin plus 9.5 μM daunomycin) into P388S and P388R cells in the presence or absence of VER (5 μg/ml) was measured at 37°C in Fischer's medium containing 10% horse serum and 25 mM HEPES. Initial uptake rates were determined as described by Skovsgaard (11). Cell pellets were dissolved in 1 N NaOH at 70°C for 1 h, neutralized, and counted in Beckman Ready-Solv cocktail by liquid scintillation counting.

For initial [3H]DAU efflux studies, cells were incubated for 10 min at 37°C in 0.7 μM DAU for P388S cells or 9.5 μM DAU for P388R cells in order to equalize the intracellular concentration of drug in both cells. Efflux was measured as described by Kessel and Wheeler (9).

RESULTS

Characterization of Multidrug Resistance in P388R Cells. The P388 subline selected in vivo for resistance to ADR when grown in tissue culture was found to be cross-resistant to other anthracyclines, such as DAU and Marcellomycin, as well as to other classes of antitumor agents, such as vinblastine, trimetrexate, and m-AMSA (Table 1). Resistance to ADR was the highest (51-fold) of the drugs tested with m-AMSA being the lowest (17-fold). Five anthrapyrazoles (see Fig. 1 for structures) were also tested for their activity against P388R cells in comparison to the sensitive P388 cells (Table 2). The degree of
the level of resistance (Table 3). P388R cells were the least resistant to PD 111,391, which was the most lipophilic of the anthrapyrazoles, while these cells were the most cross-resistant to CI-937, which was one of the least lipophilic analogues. However, partition ratios of the anthrapyrazoles were not completely predictive for their activity in P388R cells. PD 112,940 and CI-937 had virtually the same partition coefficients, but there was a 10-fold difference in the resistance of P388R cells to these compounds. While lipophilicity appears related to resistance, the affinity of the anthrapyrazoles for DNA appears to have little influence on this phenomenon (Table 3). Four of the five anthrapyrazoles had the same affinity for DNA as measured by ethidium displacement, yet varied widely in the P388R activity. PD 111,391 had the lowest DNA affinity by almost 6-fold in comparison to CI-937, yet was the best anthrapyrazole in overcoming drug resistance.

Reversal of Resistance to Anthrapyrazoles and Anthracyclines by Calcium Channel Blockers and Calmodulin Antagonists. The level of resistance of P388R cells to the anthrapyrazoles or anthracyclines could be partially reversed if the calcium channel blocker, VER (5 μg/ml), was included in the culture medium (Table 2). The degree of potentiation by VER was linearly correlated with the degree of resistance (Fig. 2). The activity of the anthrapyrazoles and anthracyclines in P388S cells was unaffected by the addition of verapamil to the medium (Table 2). Other calcium channel blockers as well as calmodulin antagonists were also tested for their ability to potentiate ADR or anthrapyrazole activity in P388R cells (Fig. 3). At the maximum tolerated nontoxic dose (1.0 μg/ml), the calmodulin antagonist W-7 had only a slight effect on ADR cytotoxicity in P388R cells and had no effect on anthrapyrazole cytotoxicity (Fig. 3). Trifluoperazine, the only other calmodulin antagonist tested in this study, was a potent potentiator of ADR and anthrapyrazole cytotoxicity in P388R cells at the same concent-

resistance of P388R cells to these compounds ranged from 2.8-fold for PD 111,391 to almost 300-fold for CI-937. PD 111,391 differs from the other members of the series in having an alcohol substitution at position 2, while the other analogues have a 2-amino substitution and a tertiary amine at position 5, whereas the other anthrapyrazoles have a primary or secondary amino substitution at position 5 (Fig. 1).

Correlation of Anthrapyrazole Lipophilicity with Lack of Cross-Resistance. A comparison of the partition ratios of the anthrapyrazoles in chloroform-water or octanol-water with the degree of resistance of P388R cells to these compounds demonstrated, in part, that the greater the lipophilicity, the lower

### Table 1 Sensitivity of cultured P388S and P388R leukemia cells to antitumor agents

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID50 (nm)</th>
<th>P388S</th>
<th>P388R</th>
<th>Degree of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daunomycin</td>
<td>110.0 ± 4.0</td>
<td>415.1 ± 555.5</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Adriamycin</td>
<td>128.8 ± 9.6</td>
<td>6586.5 ± 662.3</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Marcellomycin</td>
<td>14.5 ± 3.4</td>
<td>543.0 ± 9.7</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Vinblastine</td>
<td>3.7 ± 1.4</td>
<td>161.5 ± 5.7</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>m-AMSA</td>
<td>50.9 ± 0.8</td>
<td>851.4 ± 12.3</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Trimetrexate</td>
<td>12.2 ± 3.2</td>
<td>269.5 ± 2.7</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

* ID50 concentration that inhibits 72-h growth by 50%.
* Degree of resistance = ID50 of P388R
* ID50 of P388S

### Table 2 Potentiation by verapamil of anthrapyrazole and anthracycline activity in pleiotropically resistant P388R leukemia cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID50 (nm)</th>
<th>P388S</th>
<th>P388R</th>
<th>Degree of resistance</th>
<th>Degree of potentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD 111,391</td>
<td>163</td>
<td>178</td>
<td>463</td>
<td>328</td>
<td>2.8 ± 0.9</td>
</tr>
<tr>
<td>PD 110,911</td>
<td>127</td>
<td>114</td>
<td>628</td>
<td>437</td>
<td>4.9 ± 1.4</td>
</tr>
<tr>
<td>PD 112,940</td>
<td>141</td>
<td>145</td>
<td>3,227</td>
<td>1,014</td>
<td>23 ± 3.2</td>
</tr>
<tr>
<td>CI-942</td>
<td>498</td>
<td>468</td>
<td>75,870</td>
<td>4,069</td>
<td>152 ± 18</td>
</tr>
<tr>
<td>CI-937</td>
<td>157</td>
<td>161</td>
<td>46,781</td>
<td>1,263</td>
<td>298 ± 37</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>129</td>
<td>121</td>
<td>6,587</td>
<td>626</td>
<td>51 ± 10.5</td>
</tr>
<tr>
<td>Daunomycin</td>
<td>110,121</td>
<td>4,152</td>
<td>464</td>
<td>38</td>
<td>9.0 ± 2.5</td>
</tr>
</tbody>
</table>

* ID50 concentration that inhibits 72-h growth by 50%.
* Degree of potentiation = ID50 of P388R - verapamil
  ID50 of P388R + verapamil

### Table 3 Relationship of the lipophilic and DNA binding properties of the anthrapyrazoles with their effect on P388R cells

<table>
<thead>
<tr>
<th>Anthrapyrazole</th>
<th>Degree of resistance</th>
<th>Partition ratio</th>
<th>DNA ethidium displacement (C50, μM)</th>
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</thead>
<tbody>
<tr>
<td>PD 111,391</td>
<td>2.8</td>
<td>0.79 ± 0.6</td>
<td>142 ± 8.7</td>
</tr>
<tr>
<td>PD 110,911</td>
<td>5.0</td>
<td>0.15 ± 0.02</td>
<td>30 ± 1.0</td>
</tr>
<tr>
<td>PD 112,940</td>
<td>23</td>
<td>0.033 ± 0.001</td>
<td>27 ± 1.4</td>
</tr>
<tr>
<td>CI-942</td>
<td>152</td>
<td>0.030 ± 0.004</td>
<td>33 ± 0.75</td>
</tr>
<tr>
<td>CI-937</td>
<td>297</td>
<td>0.070 ± 0.004</td>
<td>25 ± 1.7</td>
</tr>
</tbody>
</table>

* Partition ratios were performed with 10 μg of drug per ml at the emission maximum following procedures described by Skovsgaard (33). Results are the mean of two experiments, each performed in triplicate.
* DNA ethidium displacement assays were performed as described by Baguley et al. (34).
* C50, the concentration of drug required to displace 50% of DNA-bound ethidium. Results are mean of two experiments, each performed in triplicate.
* Mean ± SE.
ANTHRAPYRAZOLE RESISTANCE

Table 5 Influx and efflux of daunomycin in P388S and P388R cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>± Verapamil (10 µg/ml)</th>
<th>Influx</th>
<th>Efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td>P388S</td>
<td>-</td>
<td>4.5 ± 0.1*</td>
<td>2.40 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>4.9 ± 0.48</td>
<td>2.15 ± 0.23</td>
</tr>
<tr>
<td>P388R</td>
<td>-</td>
<td>2.0 ± 0.9</td>
<td>16.5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2.6 ± 2.2</td>
<td>1.96 ± 0.27</td>
</tr>
</tbody>
</table>

* Influx of [3H]daunomycin was measured over the first min. Incubations were terminated by rapid chilling and centrifugation. For efflux experiments, cells were first incubated with daunomycin for 10 min, after which outward transport was measured over the initial min of efflux as described by Kessel and Wheeler (9). Influx and efflux were determined by linear regression analysis. Results are the mean of three experiments, each performed in duplicate.

Fig. 3. Comparison of the effect of calcium channel blockers and calmodulin antagonists on the potentiation of ADR, PD 119,940, and CI-937 activity in P388R cells. Cells were incubated for 72 h in the presence of drug ± the indicated potentiating agent. Columns, mean of 3 experiments, each performed in duplicate; bars, SE. *illation; DII., diltiazem; ID50, concentration that inhibits 72-h growth by 50%.

Fig. 4. Effect of VER on the net accumulation of [3H]DAU into P388S and P388R cells. Cells (2.5 × 10⁶ cells/ml) were incubated with 9.5 nM DAU (containing 0.25 nM [3H]DAU) in Fischer's medium supplemented with 10% horse serum and 25 mM HEPES ± VER (10 µg/ml) for up to 4 h. Points, mean of 2 experiments, each performed in triplicate; bars, SE.

Fig. 5. Effect of VER on DAU efflux from P388S and P388R cells. Cells were preloaded with DAU using conditions described above. Efflux was measured in the presence or absence of VER (10 µg/ml). Points, mean of 2 experiments, each performed in triplicate; bars, SE.

CONCLUSIONS

The antiarrhythmic drug, quinidine, was also found to potentiate ADR activity in P388R cells (Table 4). In addition, at nontoxic concentrations, the detergent Tween 80 potentiated ADR activity in P388R cells (Table 4). At 0.025%, Tween 80 was as effective as VER in overcoming ADR resistance. Transport Studies. The uptake and net accumulation of [3H]DAU were examined in P388S and P388R cells in the presence or absence of VER (10 µg/ml) (Table 5; Fig. 4). The initial rate of uptake (Table 5), as well as the net accumulation of DAU (Fig. 4), was significantly reduced in P388R cells compared to P388S cells. The addition of VER to the medium had no effect on the rate of uptake over the first min in either cell line, but it did partially restore DAU levels over a 4-h period in P388R cells to those observed for P388S cells. VER had no effect on either the initial uptake rate or total drug accumulation in P388S cells. In addition, no accumulation of DAU in P388S (Fig. 4) or P388R (data not shown) was observed at 4°C.

DAU efflux from P388R cells was considerably greater than that observed for P388S cells (Fig. 4). The initial rate of exodus was unaffected by VER, but VER completely reversed DAU efflux from P388R cells over the 1-h incubation to the level observed for P388S cells.
found for P388S cells (Fig. 5). As with uptake, incubation of cells in an ice bath completely blocked DAU efflux (Fig. 5).

Tween 80 (0.025%), like VER, also increased the net accumulation of DAU in P388R cells (Fig. 6). The pattern of this increase over 60 min was remarkably similar to that demonstrated for VER. The detergent had only a slight effect on DAU levels in P388S cells. Also consistent with VER, Tween 80 inhibited the efflux of DAU from P388R cells to the level of the sensitive cells (Fig. 7). The detergent had no effect on P388S efflux.

**DISCUSSION**

In the present study, we have characterized a P388 subline selected in vivo for its resistance to ADR with respect to its cross-resistance to other anticancer drugs and in terms of the reversal of resistance, particularly to the anthrapyrazoles, by calcium channel blockers and calmodulin antagonists. The P388R cell line we isolated shares many of the same characteristics of pleiotropically resistant cells previously reported (1–8). There is an accelerated efflux of DAU which is completely blocked by VER (Fig. 5; Table 5) as well as a decrease in drug uptake which is unaffected by VER (Table 5). Both of these defects have been previously reported for other models of pleiotropic drug resistance (10, 11). In our P388R cells, the failure of VER to completely reverse drug resistance (Table 2) or the net accumulation of DAU over time (Fig. 4) may be due to its inability to alter the decreased rate of drug uptake as demonstrated for DAU (Table 5), particularly since VER reduced DAU efflux in P388R cells to the level observed in P388S cells (Table 5). We also observed that, as with previous studies (10, 22), there were no differences in nuclear binding of drug between P388S and P388R cells (data not shown). Moreover, VER did not alter this binding. Thus, it is possible that the effect of VER on inward and outward drug transport in P388R could account for the partial rather than complete restoration of drug sensitivity. However, it is more likely that the mechanisms that contribute to total drug resistance are multiple in nature and more complex than merely transport defects. Chou and Yost (13), for instance, recently observed that P388/ADR cells have a faster rate of repair for ADR-induced DNA strand breaks than do normal P388 cells. Total drug resistance could be the sum of many defects, such as those observed in transport, DNA and membrane binding, and free radical formation.

Recent studies have suggested that the mechanism by which calcium channel blockers or calmodulin antagonists promote drug retention is unrelated to cellular calcium or calmodulin levels (23) or to altered calcium ion fluxes (16, 24). Several reports have suggested, instead, that the effect of VER as well as other calcium channel blockers and calmodulin antagonists may be related to their ability to interact with the plasma membrane (4, 23, 25, 26). Ramu and coworkers reported that there were differences in the membrane lipid composition (27) and membrane fluidity (28) between sensitive and multidrug-resistant P388 cells. They also noted that agents which induce alterations in the lipid domain of the plasma membrane can potentiate drug activity in resistant cells (4, 25). Tsuruo et al. (26) observed that quinidine, an agent that can alter the organization of membrane lipids, could potentiate drug activity in multidrug-resistant P388 cells. In our resistant cells, quinidine also potentiated ADR activity to almost the same degree as did VER (Table 4). Moreover, in accord with a previous study by Riehm and Biedler (29), nontoxic concentrations of the detergent Tween 80 potentiated ADR activity in P388R cells (Table 4). Tween 80 increased the net accumulation of DAU (Fig. 6) and inhibited its efflux in a manner virtually identical to that observed for VER (Fig. 7). The similarity in potentiation between Tween 80 and VER suggests that, at least for our resistant cells, calcium channel blockers and calmodulin antagonists may alter drug resistance by their interaction with the plasma membrane, perhaps in a fashion similar to that of Tween 80. Previous studies (30, 31) have demonstrated that calcium channel blockers and calmodulin antagonists can interact with the plasma membrane. The differences in potentiating activity of these agents may, in fact, be related to their membrane interactive capacity. In this regard, it is interesting to note that, of the five anthrapyrazoles tested in P388S and P388R, those with the highest lipophilicity (PD 111,391 and PD 110,911) were the most active in P388R cells, while those with the lowest partition coefficients (CI-942 and CI-937) were the least active (Table 3). This is in spite of the greater than 5-fold difference in the binding affinity for DNA between the most effective anthrapyrazole (PD 111,391) and the least effective (CI-937). The complex of multidrug resistance, it is not surprising, however, that the relationship of anthrapyrazole lipophilicity to its activity in P388R cells is less than perfect. P388R cells showed a 10-fold difference in cross-resistance to PD 112,940 versus CI-937, yet there was no significant difference in either drug’s coefficients or DNA binding affinities (Table 3). While it is unclear why the level of resistance of P388R cells to these two anthrapyrazoles is so great, it does, perhaps, serve to reinforce the multiple nature of drug resistance.

Although the P388R cells described in the present study share many of the same properties of other multidrug-resistant cells, calcium channel blockers and calmodulin antagonists may promote drug retention is unrelated to cellular calcium or calmodulin levels (23) or to altered calcium ion fluxes (16, 24). Several reports have suggested, instead, that the effect of VER as well as other calcium channel blockers and calmodulin antagonists may be related to their ability to interact with the plasma membrane (4, 23, 25, 26). Ramu and coworkers reported that there were differences in the membrane lipid composition (27) and membrane fluidity (28) between sensitive and multidrug-resistant P388 cells. They also noted that agents which induce alterations in the lipid domain of the plasma membrane can potentiate drug activity in resistant cells (4, 25). Tsuruo et al. (26) observed that quinidine, an agent that can alter the organization of membrane lipids, could potentiate drug activity in multidrug-resistant P388 cells. In our resistant cells, quinidine also potentiated ADR activity to almost the same degree as did VER (Table 4). Moreover, in accord with a previous study by Riehm and Biedler (29), nontoxic concentrations of the detergent Tween 80 potentiated ADR activity in P388R cells (Table 4). Tween 80 increased the net accumulation of DAU (Fig. 6) and inhibited its efflux in a manner virtually identical to that observed for VER (Fig. 7). The similarity in potentiation between Tween 80 and VER suggests that, at least for our resistant cells, calcium channel blockers and calmodulin antagonists may alter drug resistance by their interaction with the plasma membrane, perhaps in a fashion similar to that of Tween 80. Previous studies (30, 31) have demonstrated that calcium channel blockers and calmodulin antagonists can interact with the plasma membrane. The differences in potentiating activity of these agents may, in fact, be related to their membrane interactive capacity. In this regard, it is interesting to note that, of the five anthrapyrazoles tested in P388S and P388R, those with the highest lipophilicity (PD 111,391 and PD 110,911) were the most active in P388R cells, while those with the lowest partition coefficients (CI-942 and CI-937) were the least active (Table 3). This is in spite of the greater than 5-fold difference in the binding affinity for DNA between the most effective anthrapyrazole (PD 111,391) and the least effective (CI-937). Given the complexity of multidrug resistance, it is not surprising, however, that the relationship of anthrapyrazole lipophilicity to its activity in P388R cells is less than perfect. P388R cells showed a 10-fold difference in cross-resistance to PD 112,940 versus CI-937, yet there was no significant difference in either drug’s coefficients or DNA binding affinities (Table 3). While it is unclear why the level of resistance of P388R cells to these two anthrapyrazoles is so great, it does, perhaps, serve to reinforce the multiple nature of drug resistance.
cells described previously, differences in their response to several known potentiating agents are one notable exception. Inaba and Johnson, for example, were unable to demonstrate any drug potentiation in their resistant P388 cells by the detergent Tween 80 (1, 22). Using this same cell line, Kessel and Wilberding (32) concluded from their study that VER potentiated ADR activity in these cells by competing with ADR for exodus. While our study does not examine this possibility, the evidence with quinidine and Tween 80 supports the contention that, in P388R cells, VER and other agents potentiate drug activity by their interaction with the lipid domain of the plasma membrane. Also, unlike previous studies (14, 16, 17), NIF had no effect on the potentiation of activity of ADR or two anthrapyrazoles in P388R cells (Fig. 3). Similar discrepancies were evident for W-7. Ganapathi et al. (18) demonstrated that the calmodulin antagonist, W-13, potentiated ADR activity in ADR-resistant cells, yet W-7 in our studies had almost no drug-potentiating activity in P388R cells. It is possible that both NIF and W-7 are less potent modifiers of plasma membrane lipids than the other agents tested, or that, in the case of W-13 versus W-7, structural differences between the two compounds render the latter less effective. The differences between our cells and those of others may also serve notice to the chemotherapist that, along with the presumption of multiple defects within a given tumor cell, the circumvention of resistance is further complicated by the diversity of mechanisms which may exist between different tumors.

Finally, the present study suggests that, in the planned clinical trials with the anthrapyrazoles, patients with tumors resist-ant to ADR may also be resistant to the anthrapyrazoles, depending on the analogue being tested, but that this resistance may be at least partially reversed by potentiating agents similar to VER. The finding that the increase in ADR cytotoxicity was not observed in drug-sensitive cells indicates that the synergism may be limited to multidrug-resistant tumors.

REFERENCES

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