Genetic, Biochemical, and Cross-Resistance Studies with Mutants of Chinese Hamster Ovary Cells Resistant to the Anticancer Drugs, VM-26 and VP16-213

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ABSTRACT

The effects of the anticancer drugs 4'-demethylepipodophyllotoxin thienylidine \(\beta\)-o-glucoside and 4'-demethylepipodophyllotoxin ethylidene \(\beta\)-o-glucoside on the growth and viability of Chinese hamster ovary cells have been examined. Stable mutants which are from 4- to 12-fold more resistant to these drugs (\(Vpm^n\) mutants) are obtained in a single selection step in Chinese hamster ovary cells at a frequency of between \(0.2 \times 10^{-9}\) and \(0.5 \times 10^{-6}\). However, treatment of cells with the mutagen ethyl methanesulfonate (300 \(\mu\)g/ml for 20 hr) increased the frequency of resistant mutants by about 20- to 30-fold in different experiments. The two \(Vpm^n\) mutants which have been studied in detail exhibited significantly increased cross-resistance to a number of anticancer drugs and other compounds, including actinomycin D, Adriamycin, bruceantin, chromomycin A\(_2\), colchicine, daunomycin, ellipticine, ethidium bromide, 5-fluorouracil, trifluoracil,mithramycin, puromycin, and vinblastine. However, the level of resistance of these mutants towards several podophyllotoxin derivatives (namely, podophyllotoxin, 4'-demethylepipodophyllotoxin, and podophyllotoxin \(\beta\)-o-glucoside) and a number of other anticancer drugs, namely, 9-\(\beta\)-o-arabinofuranosyladenine, chlorambucil, hexamethylmelamine, hydroxyurea, maytansine, methotrexate, methylglyoxyl (bis)guanylylhydrazone, mitomycin C, and taxol, was found to be unaltered. Interestingly, the sensitivity of the above mutant cell lines toward a number of other anticancer drugs, namely, bleomycin, cis-diamminedichloroplatinum(II), and 1-\(\beta\)-o-arabinofuranosylcytosine, was found to be enhanced in comparison to the parental cells. The mutant cell lines showed reduced uptake of \(^3\)Hdaunomycin in comparison to the sensitive cells, and this result, together with the cross-resistance of the mutants to unrelated drugs, indicates that the genetic lesion in these mutants is most probably affecting the membrane permeability of various drugs. In cell hybrids formed between the resistant and the sensitive cells, the drug-resistant phenotype of both of the above mutants behaved in a codominant manner. The results of these studies are discussed in relation to the mechanism of action of 4'-demethylepipodophyllotoxin thienylidine \(\beta\)-o-glucoside and 4'-demethylepipodophyllotoxin ethylidene \(\beta\)-o-glucoside.

INTRODUCTION

VM-26\(^2\) and VP16-213 are 2 structurally related semisynthetic derivatives of podophyllotoxin which have proven effective against a number of different animal and human cancers (5, 21, 36, 37). In view of their favorable therapeutic responses in human clinical trials, these compounds, either alone or in combination with other drugs, are currently being used in the treatment of a number of human cancers, including Hodgkin’s and non-Hodgkin’s lymphomas, leukemias, brain tumors, bladder carcinomas, and small-cell bronchogenic carcinomas (3, 5, 18, 25, 27-29, 31). Although VM-26 and VP16-213 are derivatives of podophyllotoxin (see Chart 1 for chemical structures), their mechanism of action appears to be quite different from that of the parental compounds podophyllotoxin and 4'-demethylepipodophyllotoxin. While the latter compounds inhibit microtubule assembly and arrest cells during mitosis, VM-26 and VP16-213 have no effect on microtubule assembly, and cells treated with these drugs are blocked in a premitotic phase (late S or G\(_2\)) of the cell cycle (20, 22, 23, 26, 36, 38). Earlier studies on the effects of VM-26 and VP16-213 in mammalian cells indicate that treatment with these compounds causes a reduction in nucleoside uptake and preferential inhibition of thymidine incorporation into DNA (8, 9, 23). In human hematopoietic cell lines exposed to VP16-213, a high incidence of chromosomal aberration has been reported by Huang et al. (19). More recently, Loike and Horowitz (24) have observed that, in HeLa cells treated with VP16-213 or VM-26, cellular DNA is converted to a lower-molecular-weight form, suggesting that these compounds probably induce single-strand lesions in the DNA. Although all of the above observations indicate that the primary effect of these compounds is on DNA metabolism, their precise mode of action remains unclear at present.

To understand the mechanisms of action of various cytotoxic chemotherapeutic drugs, we have been using a genetic approach in which cellular mutants resistant to various agents are selected and subsequently characterized genetically and biochemically. Using this approach, recently mutants of CHO cells exhibiting greatly increased resistance towards podophyllotoxin and which were affected in a microtubule-associated protein were described (10, 14). The various podophyllotoxin-resistant mutants which were examined exhibited proportionally increased resistance towards various podophyllotoxin analogues which possessed microtubule-inhibitory activity. However, these mutants did not show any cross-resistance toward VM-26 or VP16-213, thus providing further evidence that the mechanism of action of these compounds is different from that of the parental podophyllotoxin-like compounds (12, 14). In the present paper, we describe the selection and partial characterization of mutants of CHO cells which show stable, high levels of resistance toward VM-26 and VP16-213 (\(Vpm^n\) mutants). The results of cross-resistance studies with 2 of the \(Vpm^n\) mutants, which behave codominantly in cell hybrids, toward several podophyllotoxin derivatives and a wide variety of other antineoplastic drugs are also presented in this paper.
The dishes were incubated for 6 to 7 days at 37°, after concentration to that obtained in the absence of the drug. The cross-determined as the ratios of the number of colonies at a given drug

The relative plating efficiencies (the same as cloning efficiency) were calculated as described above. The D_{10} value of a drug towards a cell line refers to the dose of the drug which reduces relative plating efficiency of a cell line to 10% of that obtained in the absence of any drug.

Selection of Mutants. Selection of mutants was carried out by procedures similar to those used earlier (11, 16–18). The WT CHO cells were seeded at a concentration of about 1 x 10^6 cells on 100-mm-diameter dishes. After about 1 to 2 days, when cells were exponentially growing and when the dishes were nearly one-fourth to one-third confluent, one of the dishes was treated with 300 µg of the mutagen ethyl methanesulfonate per ml for 20 hr. Another dish which served as control received an equal amount of the solvent (growth medium). The above treatment with the mutagen results in about 50% cell killing (10, 16, 17). The mutagen-treated and control cells were grown for 3 days in nonselective medium to allow time for mutation fixation (33). The selection of mutants was carried out by plating 5 x 10^5 cells/100-mm-diameter dish on several dishes in medium containing different concentrations of either VM-26 or VP16-213. The plating efficiencies of the cells at the time of plating were determined by plating a known number of cells in nonselective medium, and the mutation frequencies observed were calculated based upon the number of viable cells which were plated in the selection medium.

Cellular Uptake of [^3H]Daunomycin. For studying the cellular uptake of [^3H]daunomycin, about 5 x 10^5 cells were seeded (in duplicate for each time period) into the wells of 24-well tissue culture dishes. After about 2 days, when the dishes were nearly confluent, the medium was carefully aspirated, and 0.25 ml of a solution of desired concentration of [^3H]daunomycin in growth medium was added to each well. After the indicated period of uptake, labeled medium was removed, and cells were rinsed 3 times with phosphate-buffered saline. The cells from each well were dissolved in 0.25 ml of a solution of 0.4% deoxycholic acid in 0.1 n NaOH, and the amount of radioactivity was measured after the addition of 3 to 4 ml of aqueous counting scintillant (Amersham/Searle Corp., Arlington Heights, Ill.). At the same time, total numbers of cells in 2 parallel control wells of each cell line were determined by trypsinization and the counting of aliquots in a Coulter electronic counter. The uptake of [^3H]daunomycin in different cell lines was normalized for a constant cell number.

RESULTS

Effect of VM-26 and VP16-213 on the Growth and Viability of CHO Cells. The effects of VM-26 and VP16-213 on cell growth and division in a number of mammalian cell lines other than CHO have been reported earlier (8, 9, 22, 26, 36, 37). However, as a first step to selecting mutants resistant to VM-26 and VP16-213 in CHO cells, the effect of treatment with these chemicals on the growth and viability of CHO cells was examined. Chart 2 shows the effect of treatment with 3 different concentrations of VP16-213 on the growth of CHO cells. At a concentration of 0.5 µg/ml, VP16-213 reduced the growth rate of CHO cells initially to about one-half as compared to the control untreated cells; however, at later times, the rate of cell growth slowed down considerably and ceased nearly completely by 72 hr. At 1.0 µg/ml, the rate of cell growth was diminished greatly from the very beginning and, after a small initial increase in cell number (=50%), cell growth completely stopped. At a 10-µg/ml dose of VP16-213, virtually no increase in cell number was observed. Treatment with VM-26 affected the growth of CHO cells in a manner very similar to that observed with VP16-213; however, in this case, similar effects on cell growth were observed at concentrations which were 10-fold lower than those of VP16-213 (Chart 2).

The effect of treatment with various concentrations of VP16-213 and VM-26 on the viability of CHO cells, as measured by their cloning efficiency, was also determined in the above experiment, and the results of these studies are shown in Chart 3. As can be seen, treatment of cells with either VM-26 or VP16-213 led to a rapid decline in the cloning ability of CHO cells. At all of the concentrations of the drugs which were examined, the decrease in cell viability was found to occur exponentially with
The above experiments indicated that VM-26 and VP16-213 behaved similarly with regard to their effects on cell growth and viability. However, of these 2 drugs, since VM-26 was effective at much lower concentrations, it was used for the selection of resistant mutants initially. To select mutants resistant to VM-26, the plating efficiency of WT cells in medium containing different concentrations of the drug was determined (Chart 4). As seen from the dose-response curve, there was a sharp decline in the plating efficiency of CHO cells at drug concentrations between 0.01 and 0.05 μg/ml, and in the presence of 0.1 μg/ml VM-26, the plating efficiency was reduced to less than 1 in $10^5$ (results not shown). However, when larger number of cells were plated in the presence of 0.1 μg/ml VM26, a few colonies were observed in various dishes. The frequencies of such colonies in nonmutagenized cultures in 3 separate experiments was found to be in the range of 0.2 to $0.5 \times 10^{-5}$. The prior treatment of WT cells with the chemical mutagen ethyl methanesulfonate led to a 20- to 30-fold increase in the frequencies of resistant colonies, as compared to the parallel untreated cultures (results not shown).

Some of the colonies which appeared in the above experiments were picked at random and were grown in the nonselective medium. Subsequently, the degree of resistance of these clones toward VM-26 was examined. All 6 colonies which were examined in this way proved more resistant to VM-26 as compared to the WT cells, and the dose-response curves of 4 different clones (Vpm*-1, -3, -5, and -6) toward VM-26 are shown in Chart 4. Based upon their $D_{50}$ values (concentrations of the drug which reduce plating efficiency of the cell line to 10% as compared to that observed in the absence of any drug) for VM-26, these cell lines are between 4- and 12-fold more resistant to the drug than were the parental WT cells.

The dose-response curves of the WT and the above mutant cell lines toward VP16-213 are shown in Chart 5. As can be seen, all of the mutant cell lines also exhibit increased resistance toward VP16-213, and the level of resistance in every case was very similar to that observed for VM-26. These results indicate that all of the above mutants behave similarly toward both VM-26 and VP16-213 and that it should be possible to select similar mutants using VP16-213 instead of VM-26. In accordance with this, in one experiment in which cells were plated separately in the presence of either VM-26 (0.1 μg/ml) or VP16-213 (1.0 μg/ml)
Compounds 3 (1, 2, 35). As can be seen (Chart 6, ß to D), both of the mammalian cell mutants which are affected in the membrane permeability of drugs exhibit increased resistance to these compounds (see Chart 5). Both VM-26 and VP16-213 (12, 14, 20, 23). The cross-resistance of the Vpm" mutants to various podophyllotoxin analogues, e.g., 4'-demethylepipodophyllotoxin and podophyllotoxin-|3-D-gluco-side, which have been examined (see Table 2). The lack of cross-resistance toward other podophyllotoxin analogues, e.g., 3-APRIL 1983

Cellular Resistance to VM-26 and VP16-213

of 1 month. For 2 of the cell lines, vpm"-1 and Vpm"-5, which have been further investigated, their levels of resistance toward VM-26 and VP16-213 have remained unchanged for more than 1 year.

Cross-Resistance of Vpm" Mutants to Various Anticancer Drugs and Other Inhibitors. In our earlier work with mammalian cell mutants resistant to various drugs, e.g., emetine, ouabain, and podophyllotoxin, we have observed that the cross-resistance of mutants toward other drugs and inhibitors can provide valuable information regarding the nature of the genetic lesions in mutants and the mechanism of action of a given drug (10–12, 14, 15). In view of this, the cross-resistance of 2 of the Vpm" mutants (Vpm"-1 and Vpm"-5) toward a variety of drugs and inhibitors was investigated.

The results of cross-resistance studies with the above Vpm" lines toward podophyllotoxin, colchicine, daunomycin, and puromycin are shown in Chart 6. Podophyllotoxin was investigated because both VM-26 and VP16-213 are derived from this compound (see Chart 1), although unlike podophyllotoxin these do not inhibit microtubule assembly (20, 21, 23). As can be seen (Chart 6A), the sensitivity of the 2 Vpm" mutants toward podophyllotoxin remained unchanged and was similar to that of the parental WT line. The above mutants do not also exhibit any cross-resistance toward other podophyllotoxin analogues, e.g., 4'-demethylepipodophyllotoxin and podophyllotoxin-ß-D-glucoside, which have been examined (see Table 2). The lack of cross-resistance of Vpm" mutants to various podophyllotoxin derivatives, which inhibit microtubule assembly, is consistent with the fact that the mechanism of action of these compounds is different from that of VM-26 and VP16-213 (12, 14, 20, 23). The cross-resistance of mutants toward colchicine, daunomycin, and puromycin was examined because earlier studies indicate that many of the mammalian cell mutants which are affected in the membrane permeability of drugs exhibit increased resistance to these compounds (1, 2, 35). As can be seen (Chart 6, B to D), both of the Vpm" mutants were found to be somewhat more resistant to colchicine, daunomycin, and puromycin. The level of cross-resistance of the Vpm"-1 mutant to these drugs was slightly higher in comparison to the Vpm"-5 mutant, although the latter is more resistant to both VM-26 and VP16-213.

The cross-resistance of the Vpm" mutants to the above drugs indicated that the genetic lesions in these mutants were perhaps affecting the cellular uptake of various compounds. This possibility was directly tested by examining the cellular uptake of [3H]daunomycin by various cell lines, and the results of these studies are presented in Table 1. As can be seen, in comparison to the WT cells, both of the mutant cell lines showed a somewhat reduced uptake of [3H]daunomycin. At 60 min, the amount of [3H]daunomycin that was taken up by the mutant cell lines was approximately one-half of that observed with the WT cells. The reduced uptake of [3H]daunomycin in the mutant cell lines strongly suggests that the biochemical lesions in these mutants affect the cellular permeability of various drugs.

Both VM-26 and VP16-213, either alone or in combination with other drugs, are currently being clinically used in the treatment of various types of cancer (3, 18, 25, 27–29, 31). Since development of drug resistance of tumor cells is one of the major

Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Duration of uptake (min)</th>
<th>[3H]Daunomycin uptake/10^6 cells (cpm)</th>
<th>Uptake relative to WT cells</th>
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<tr>
<td>WT</td>
<td>30</td>
<td>2107 ± 120^a</td>
<td>100</td>
</tr>
<tr>
<td>Vpm&quot;-1</td>
<td>30</td>
<td>1435 ± 70</td>
<td>68.1</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>4319 ± 185</td>
<td>100</td>
</tr>
<tr>
<td>Vpm&quot;-5</td>
<td>30</td>
<td>1898 ± 140</td>
<td>43.9</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1627 ± 146</td>
<td>77.2</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2248 ± 215</td>
<td>52.0</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

2 R. S. Gupta, unpublished results.
problems in cancer chemotherapy (4, 34), it was of much interest to determine if the Vpm* mutants would exhibit cross-resistance to other anticancer drugs. The anticancer drugs which have been examined include those which directly interact with DNA (e.g., actinomycin D, Adriamycin, bleomycin, chromomycin A3, cisplatin, daunomycin, ellipticine, mithramycin, and mitomycin C), several inhibitors of microtubule function and other cellular processes (e.g., vinblastine, maytansine, nocardazole, taxol, and bruceantin), various base analogues and anti-metabolites (e.g., 9-β-D-arabinofuranosyladenine, ara-C, 5-fluorouracil, fluracil, hydroxyurea, methotrexate, methyl-GAG and 6-thioguanine), and alkylating agents such as chlorambucil and hexamethylmelamine.

Many of these drugs, e.g., bruceantin, fluracil, ellipticine, nocardazole, and taxol, are currently being investigated as experimental anticancer drugs by the National Cancer Institute (6). The results of the cross-resistance studies of the Vpm* mutants for these and some other drugs are summarized in Table 2. From the results presented in Table 2, it is clear that the mutants selected for resistance to VM-26 and VP16-123 exhibit between 2- and 2.5-fold increased resistance toward Adriamycin, daunomycin, and ellipticine. The 2 mutants also exhibited small but significantly increased resistance (between 1.3- and 1.8-fold) toward actinomycin D, bruceantin, chromomycin A3, 5-fluorouracil, fluracil, mithramycin, and vinblastine. The pattern of resistance of the 2 mutants was similar for all of the above drugs, except possibly for bruceantin, to which only the Vpm*-1 mutant showed somewhat increased resistance. In contrast to the above anticancer drugs, the 2 Vpm* mutants were found to have become somewhat more (about 1.5- to 3-fold) sensitive to ara-C, bleomycin, and cis-platin, in comparison to the parental WT cells. For the remaining drugs which are listed in Table 2, the sensitivity of the Vpm* mutants remained unchanged and was very similar to that observed for the WT cells.

Behavior of Vpm* Mutants in Cell Hybrids. To find out whether the drug-resistant phenotype of the Vpm* mutants behaved recessively or dominantly in a Vpm* x Vpm* situation, somatic cell hybrids were constructed between the 2 Vpm* mutants and a sensitive cell line EOT-3 (10, 13). This latter cell line, which is derived from the WT cells, has been selected for resistance to ouabain and is also unable to grow in hypoxanthine-aminopterin-thymidine-supplemented medium due to a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase. The hybrids between the EOT-3 cell line and the Vpm* mutants can then be selected in hypoxanthine-aminopterin-thymidine medium supplemented with 2 x 10^-3 M ouabain in which none of the parental cell lines grow (10-13). The cell fusion between the 2 Vpm* mutants and the EOT-3 cell lines (i.e., Vpm*-1 x EOT-3, Vpm*-5 x EOT-3, and WT x EOT-3) was carried out in the presence of polyethylene glycol and dimethyl sulfoxide by procedures which have been described earlier (10, 11). Hybrid clones were obtained in the above crosses with very high frequencies (10^-2 to 10^-3). In contrast, no colonies were observed in the control self-crosses (i.e., Vpm*-1 x Vpm*-1, Vpm*-5 x Vpm*-5, and EOT-3 x EOT-3), even when up to 2 x 10^6 cells were plated in the above medium. Some of the clones (4 from each cross) which grew in the selective medium in different crosses were picked and their hybrid nature, i.e., pseudotetraploid karyotype, was ascertained by karyotype analysis (results not shown). Subsequently, the degree of resistance of various hybrid and parental cell lines toward VM-26 was determined (Chart 7). As can be seen, all hybrid cell lines obtained after fusing Vpm*-1 or Vpm*-5 cells with the EOT-3 cell line show slightly higher levels of resistance toward VM-26 in comparison to the sensitive cell lines. In contrast, the hybrids formed between the 2 sensitive lines (WT x EOT-3) which served as a control in these experiments showed the same degree of resistance toward VM-26 as did the parental lines. These results indicate that the drug-resistant phenotype of the Vpm* mutants behaves codominantly in cell hybrids.

### DISCUSSION

Results presented in this paper show that in CHO cells, stable mutants which are up to 12-fold more resistant to the anticancer drugs VM-26 and VP16-213 can be readily obtained in a single selection step. The frequency of resistant cells in the CHO cell line was in the range of 0.2 to 0.5 x 10^-6; however, prior treatment of cells with the mutagen ethyl methanesulfonate greatly increased the frequency of resistant colonies, which is...
mutants examined showed somewhat enhanced sensitivity toward ara-C, bleomycin, and cis-platin. One of the drugs which has been frequently used in combination with VP16-213 (or VM-26) is Adriamycin, and since resistance to both of these drugs could develop in a single step, this drug combination may not prove very effective on a long run. On the other hand, drug combinations in which mutants resistant to one drug show enhanced sensitivity to the other (e.g., VM-26 or VP16-213 and cis-platin) could prove particularly useful in chemotherapy. In this regard, recent reports which describe that the use of a combination of cis-platin and VM-26 in treating patients with either small-cell carcinoma of the lung or neuroblastomas produced much better results than did treatment with either of these 2 drugs alone are of much interest (18, 31).

At present, the mechanism of cellular toxicity of VM-26 and VP16-213, both of which produced similar effects and hence apparently act in an identical manner, is not well understood. Although these compounds are derivatives of the microtubule inhibitor podophyllotoxin, unlike the parental compounds, these drugs do not inhibit microtubule assembly, and mutants resistant to podophyllotoxin do not exhibit any cross-resistance to these compounds (12, 14). The lack of cross-resistance of VpmR mutants to various podophyllotoxin analogues (e.g., 4'-demethyl-yleipodophyllotoxin, podophyllotoxin-β-o-glucoside, and deoxypodophyllotoxin), which possess microtubule-inhibitory activity, suggests that the cellular uptake of these 2 types of compounds do not occur by a common mechanism. In an earlier study, Grieder et al. (9) have compared the early effects of VM-26 and VP16-213 on cultured mastocytoma cells with those of many other cytostatic agents, including podophyllotoxin-β-o-benzylidene glycoside, colchicine, vincristine, bleomycin, methotrexate, 1,2-bis(3,5-dioxopiperazin-1-yl)propane (ICRF-159), mechlorethamine, ara-C, 6-mercaptopurine, and X-rays. On the basis of their studies, these authors concluded that of these agents, only the cellular effects induced by X-rays were similar to those seen with VM-26 or VP16-213. The similarity between the effects of X-rays and these drugs is also observed in human clinical studies, because Fontana (7) has recently reported a radiation recall that is associated with VP16-213 therapy. This analogy between the effects of X-rays and VM-26 or VP16-213 is further extended in the present study. For example, the kinetics of cell killing by both VM-26 and VP16-213, which occur without any initial lag (Chart 3), is very similar to that of X-rays but differs from that of other cytotoxic agents, e.g., emetine, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole, trichodermin, and UV, the effects of which have been examined in earlier studies (16, 17, 32). Recently, the mutagenic effects of VM-26, VP16-213, and X-rays on mutation induction at 5 independent genetic loci in CHO cells was investigated (32, 33). Results of these studies showed that like X-rays, which induced mutations at the hypoxanthine-guanine phosphoribosyltransferase locus (confering resistance to 6-thioguanine) but produced no mutagenic response at the other genetic loci affecting essential gene functions, both VM-26 and VP16-213 also showed a strong mutagenic response at the hypoxanthine-guanine phosphoribosyltransferase locus, and the other genetic loci examined showed either no response or a very weak response. In the case of X-rays, the predominant types of genetic lesions produced are the single- and double-stranded DNA breaks, and it is of much interest that similar DNA strand breaks are also produced in HeLa cells upon exposure to either

consistent with their mutational origin. Although the biochemical alterations in mutant cells have not yet been completely identified, cross-resistance of the mutants to unrelated compounds such as colchicine, puromycin, ethidium bromide, etc., strongly suggests that the lesion in these mutants most probably affects the membrane permeability of various drugs. The reduced uptake of [3H]daunomycin in the mutant cell lines as compared to the parental WT cells provides further support for the above inference. In cell hybrids constructed between 2 resistant mutants (VpmR-1 and VpmR-5) and a sensitive cell line, the drug-resistant phenotype of the mutants was expressed in a codominant manner, as indicated by the increased resistance of hybrid cell lines to these drugs. The codominant behavior of the VpmR mutants suggests that in contrast to some of the recessive mutants, which can be readily selected only in pseudodiploid cell lines such as the CHO cell line (13), the above type of mutant may also readily develop in diploid cells.

Both VP16-213 and VM-26, either alone or in combination with one or more of the other drugs (e.g., Adriamycin, bleomycin, cis-platin, ara-C, cyclophosphamide, procarbazine, and hexamethylmelamine), have proven useful in the treatment of a number of different types of human cancer (3, 18, 25, 27-29, 31).

The reduced uptake of various drugs such as colchicine, puromycin, ethidium bromide, etc., strongly suggests that the lesion in these mutants most probably affects the membrane permeability of various drugs. The reduced uptake of [3H]daunomycin in the mutant cell lines as compared to the parental WT cells provides further support for the above inference. In cell hybrids constructed between 2 resistant mutants (VpmR-1 and VpmR-5) and a sensitive cell line, the drug-resistant phenotype of the mutants was expressed in a codominant manner, as indicated by the increased resistance of hybrid cell lines to these drugs. The codominant behavior of the VpmR mutants suggests that in contrast to some of the recessive mutants, which can be readily selected only in pseudodiploid cell lines such as the CHO cell line (13), the above type of mutant may also readily develop in diploid cells.

Both VP16-213 and VM-26, either alone or in combination with one or more of the other drugs (e.g., Adriamycin, bleomycin, cis-platin, ara-C, cyclophosphamide, procarbazine, and hexamethylmelamine), have proven useful in the treatment of a number of different types of human cancer (3, 18, 25, 27-29, 31). Since the prevention of drug resistance is one of the main reasons for using combination chemotherapy, our studies on the cross-resistance of VpmR mutants to other anticancer drugs provide valuable information regarding the drug combinations to which resistance may develop simultaneously in a single step. Studies presented in this paper show that of the various anticancer drugs examined, mutants resistant to VM-26 and VP16-213 exhibit small (between 1.3- and 2.2-fold) but significant cross-resistance to actinomycin D, Adriamycin, bruceantin, chromomycin A₃, colchicine, daunomycin, ellipticine, 5-fluorouracil, florafur, mithramycin, and vinblastine. Mutants resistant to some of these drugs, e.g., actinomycin D, Adriamycin, colchicine, and vinblastine, which exhibit cross-resistance to each other, have been reported previously in mammalian cell lines (1, 2, 30, 34, 35), indicating that the cellular transport of these drugs may involve a common pathway. In contrast to the above drugs, the VpmR mutants showed no change in their degree of resistance toward 9-β-α-arabinofuranosyladenine, chlorambucil, hexamethyldiamine, hydroxyurea, maytansine, methotrexate, methyl-GAG, mitomycin C, and taxol. Most interestingly, the 2 VpmR
VM-26 or VP16-213 (19, 24). These results suggest that the genetic lesions produced by VM-26 and VP16-213 are probably very similar to those produced by X-rays.

ACKNOWLEDGMENTS

I am much indebted to Mitra Boodram, Rajni Gupta, and Dawn Kirkconnell for the excellent technical assistance. I am also very thankful to various investigators and agencies for providing many of the drugs which have been used in these studies.

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