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Characterization of Drug Resistance to VM-26 in A2780 Ovarian Carcinoma Cells

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Chun-Mao Lin, Tzong-Yueh Chen, Leng-Fang Wang, Cho-Fat Hui and Jaulang Hwang (2001) Characterization of drug resistance to VM-26 in A2780 ovarian carcinoma cells. *Zoological Studies* **40**(1): 71-78. Human ovarian carcinoma A2780 cells resistant to VM-26, a topoisomerase II-targeting drug, were cloned. Cross-resistance test showed that the resistant cells were 300 fold more tolerant toward VM-26 than were parental cells, whereas tolerance towards other drugs increased only 3 to 10 fold. VM-26 triggered apoptosis in a variety of cells including rodent cells, but not in resistant cells. This resistance might not be due to multidrug resistance (MDR). The topoisomerase II mRNA levels showed only a slight variation between VM-26-treated and -untreated resistant cells, and the difference in protein levels was about 2 fold. This implies that the level of topoisomerase IIβ expression may be unrelated to drug resistance. The DNA strand-passing activity of topoisomerase II affected by VM-26 was measured by K-SDS precipitation of the topoisomerase II-DNA complex, and topoisomerase II decatenation of kinetoplastic DNA (K-DNA). The results showed that the VM-26 influence on topoisomerase II cleavable activity was much less in resistant cells. Alteration of drug targeting sites in topoisomerase II might be a factor contributing to VM-26 drug resistance in these resistant cells.

Key words: Topoisomerase II, Drug resistance, VM-26.

The topological states of DNA are closely related to DNA functions. Two fundamentally different types of DNA topoisomerases, topoisomerase I and topoisomerase II, have been found in nature. These 2 topoisomerases can break and rejoin 1 or 2 strands, respectively, of double-stranded DNA. Through these 2 different mechanisms, the topological states of DNA can be modulated, and the important biological processes, such as DNA replication, recombination, transposition, nucleosome assembly, and transcription, can then be performed (Uemura and Yanagida 1984, Adachi et al. 1991, Wang 1996).

Recently, DNA topoisomerases have been identified as important therapeutic targets in cancer chemotherapy (Hsiang et al. 1985, Lock and Ross 1987, Kaufmann 1991). Camptothecin is a topoisomerase I-targeting antitumor drug, and VM-26 (teniposide) is a topoisomerase II-targeting antitumor drug (Liu 1989). The identification of topoisomerases as antitumor therapeutic targets has offered new insights into the possible cell-killing mechanisms of anticancer drugs such as VM-26. The cytotoxic action of VM-26 is initiated by trapping the topoisomerase II-DNA cleavable complex, followed by induction of DNA fragmentation that leads to cell death (Zhang et al. 1990). Although treatment with these drugs has resulted in the amelioration of some types of human cancer, cancer patients always develop drug resistance after a certain period of treatment. The development of drug resistance appears to be a major impediment to the successful chemotherapy of human tumors. Thus, identifying the mechanisms that confer drug resistance might help resolve these problems in cancer chemotherapy.

Several possible mechanisms that can confer

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drug resistance have been considered, including drug transportation (Larsen and Skladanowski 1998, Ma et al. 1998), drug metabolism (Kramer et al. 1988, Bellamy et al. 1989), drug target defect (Hsiung et al. 1996), and decrease in the drug target (Kawanami et al. 1996). In the drug transportation mechanism, cell lines carrying the multidrug-resistance (MDR) phenotype display cross-resistance to multiple structurally unrelated drugs such as anthracyclines, vinca alkaloids, epipodophyllo-toxins, actinomycin D, colchicine, and adriamycin. Cells with the MDR phenotype show a decrease in drug accumulation and an overexpression of a cell membrane glycoprotein (Bradley et al. 1988), designated P170 (Pgp), which functions as an energy-dependent efflux pump. Resistance to these drugs can be reversed by verapamil, a calcium channel blocker, which acts as a competitive inhibitor of Pgp-mediated drug efflux. In the drug metabolism mechanism, glutathione S-transferases (GSTs), isozymes of a multigene family, catalyze the conjugation of glutathione (GSH) to a variety of electrophilic compounds as the first step in a detoxification pathway. Therefore, it has been suggested that the definition of the MDR phenotype should be broadened to include the GSH redox cycle (Kramer et al. 1988, Bellamy et al. 1989, Tew et al. 1998). In the drug target defect mechanism, VM-26 has been shown to trap the topoisomerase II-DNA complex. When decreased expression or mutations occur in topoisomerase II, VM-26 can no longer recognize its binding site, thus giving rise to drug resistance (Hsiung et al. 1996).

The purpose of this study was to isolate and characterize VM-26-resistant cells, and through the study of a variety of characteristic changes between parental and drug-resistant cells, we hope to uncover the possible mechanisms of cellular resistance to VM-26.

MATERIALS AND METHODS

Cell line

Human ovarian A2780 carcinoma cells were cultured using RPMI-1640 containing 10% fetal bovine serum, penicillin/streptomycin (50 IU/ml and 50 μg/ml, respectively), and 1 mM glutamine in the presence of 5% CO_2 at 37 °C.

Chemicals

VM-26 was a gift from Dr. Leroy F. Liu (Robert Wood Johnson Medical School, NJ, USA). Cell culture medium, serum, and chemicals (penicillin, streptomycin, and glutamin) were purchased from GIBCO/BRL (Grand Island, NY, USA); colchicine, adriamycin, camptothecin, and m-AMSA were from Sigma (St. Louis, MO, USA). All radioisotopes (^{3}H-thymidine, ^{35}S-methionine, and ^{32}P-dNTP) were from Amersham (Buckinghamshire, UK). The topoisomerase II assay kit was from TopoGEN (Columbus, OH, USA).

VM-26 (teniposide)-resistant cell selection

VM-26 (MW = 656.67) at 10 ng/ml was included in the culture medium until resistant colonies formed. The resistant colonies were trypsinized and enriched, then divided into 2 groups, one of which was cultured in verapamil-containing medium (10 μg/ml), and the other with increasing VM-26 concentrations in the medium up to 40 ng/ml. When new resistant colonies formed, stepwise increase of VM-26 concentration in the cultures was performed.

Cross-resistance test

To study cross-resistance to colchicine, m-AMSA, camptothecin, adriamycin, and vinblastine, parental A2780 cells and resistant cells were treated with these drugs individually for 72 h in 24-well plates, and the ID_{50} was determined as described (Chow et al. 1989).

DNA fragmentation assay

Cells (2×10^5 cells/ml) with various treatments were harvested and lysed with 100 μl lysis buffer (50 mM Tris-HCl, 10 mM EDTA, 0.5% N-lauroylsarcosine, 0.5 mg/ml proteinase K) at 50 °C for 3 h, and ribonuclease A (0.5 mg/ml) was added for a further 1-h incubation. DNA samples were extracted with phenol/chloroform twice. Electrophoresis was carried out on a 1.8% agarose gel in TBE buffer (45 mM Tris-borate and 1 mM EDTA, pH 8.0). DNA ladders were visualized by staining with ethidium bromide and photographed.

Western blot analysis of topoisomerases

Total cellular proteins were extracted with extraction solution (1 mM PMSF, 50 μg/ml aprotinin, 30 mM tetrasodium pyrophosphate, 50 mM sodium fluoride, 100 mM sodium orthovanadate, 150 mM sodium chloride, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, 50 mM Tris-

HCl, pH 8.0) by repeated vortexing. Supernatant was collected after centrifugation, and the protein concentration was determined. Protein at 100 μg was loaded in each well for 7.5% SDS-PAGE. For Western blotting, proteins were transferred from polyacrylamide gel to polyvinylidene difluoride membranes. Membranes were probed with rabbit anti-human topoisomerase II polyclonal antisera (Hwang et al. 1989), and then reacted with anti-rabbit IgG alkaline phosphatase conjugate antibody as the secondary antibody. Color was developed in a reaction with BCIP (5-bromo-4-chloro-3-indolylphosphate) and NBT (nitro blue tetrazolium).

KCl/SDS precipitation assay of trapped topoisomerase II-DNA complex by VM-26 treatment

The in vivo formation of the covalent topoisomerase II-DNA complex was measured using K-SDS precipitation (Chen et al. 1990). Cellular DNA was labeled by adding ^{3}H-thymidine into the medium to a final concentration of 10 μCi/ml for 8 h. Then 10^5 cells/well in a 24-well plate were plated and treated with various concentrations of the drug for 60 min. The medium was removed from each well, and cells were washed with PBS and lysed with 1 ml of prewarmed (65 °C) lysis solution (1.25% SDS, 5 mM EDTA, 0.4 mg/ml salmon sperm DNA). Lysate was transferred to a centrifuge tube and sheared using a 21-gauge needle. The samples for background control were treated the same way as in the above procedure but 400 μg/ml of proteinase K was added to the lysis buffer, and incubation was for 2 h at 50 °C. KCl (325 mM) at 250 μl was added, vortexed vigorously, cooled on ice for 10 min, and centrifuged at 2500 rpm in a Beckman RT6000 centrifuge for 10 min at 4 °C. The pellet was washed twice in 1 ml of wash solution (10 mM Tris-HCl, 100 mM KCl, 1 mM EDTA, 0.1 mg/ml salmon sperm DNA), incubated at 65 °C for 10 min, cooled on ice, then centrifuged at 2500 rpm for 10 min. The pellet was resuspended in 400 μl prewarmed H_2O (65 °C) and combined with 4 ml of scintillation liquid, and the radioactivity counts determined.

Nuclear extract preparation

Cells were harvested and washed in Hypo solution (5 mM potassium phosphate pH 7.0, 2 mM $MgCl_2$, 1 mM DTT), then resuspended in Hypo solution containing 0.2% NP-40, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.1 μg/ml of aprotinin. Nuclei were isolated by centrifugation at 12 000 rpm for 10 min. The nuclear pellet was resuspended in Hypo solution containing 2 mM PMSF and 0.2 μg/ml aprotinin, then 5 M NaCl was added to achieve a final concentration of 0.35 M. After 60 min on ice, the solution was centrifuged at 12 000 rpm for 15 min. Supernatant was diluted with an equal volume of Hypo solution containing 20% glycerol, aliquoted to 100 μl/tube, and then stored at –70 °C.

Topoisomerase II strand-passing activity assay in isolated nuclear extract

The topoisomerase II strand-passing activity in the nuclear extracts was assayed using the kinetoplast decatenation assay (Muller et al. 1989). Kinetoplast-catenated DNA (K-DNA) at 0.2 μg was incubated with nuclear extract protein in the reaction mixture (50 mM ATP, 0.5 mM dithiothreitol, 30 μg/ml BSA) for 10 min at 30 °C. The reaction was stopped by adding 5x stop solution (5% Sarkosyl, 0.0025% bromophenol blue, 25% glycerol). Then the reaction mixtures were loaded onto 1% agarose gels containing 0.5 μg/ml ethidium bromide. After electrophoresis, the gel was destained and photographed.

RESULTS

Isolation of VM-26-resistant cells and cross-resistance test

So far, we have obtained sublines that are VM-26 resistant up to a concentration of 2 μg/ml. The subline selected at a concentration of 80 ng/ml VM-26, designated R80, was used for subsequent studies. We tested the cross-resistance of R80 towards colchicine, adriamycin, vinblastin, camptothecin, m-AMSA, or VM-26 using concentrations from 0.1 to 10 μg/ml. The results showed that VM-26-resistant cells were more tolerant towards the drugs tested than were the parental cells: 3-fold for adriamycin; 10-fold for colchicine, camptothecin, and m-AMSA; and 300-fold for VM-26 (Fig. 1). Therefore, this resistance towards VM-26 might not be due to multidrug resistance.

R80-resistant cells resistant to VM-26-triggered apoptosis

Apoptosis was triggered in mammalian A2780 cells, and rodent CHO and NIH3T3 cells by treatment with VM-26. DNA fragmentation of these cells was induced in a dose-dependent manner. Cells were treated with various concentrations of VM-26

(0, 5, 10, and 30 μM) for 24 h, and genomic DNA was isolated and analyzed. Apoptosis could be triggered in A2780 (Fig. 2A), CHO (Fig. 2B), and NIH3T3 (Fig. 2C) cells by VM-26. However, R80-resistant cells showed no DNA fragmentation with VM-26 treatment (Fig. 2A).

Protein level of topoisomerase II not significantly affected by VM-26 treatment in resistant cells

Differences in topoisomerases IIβ protein expression levels between parental and resistant cells were studied using rabbit anti-topoisomerase II polyclonal antisera (Hwang et al. 1989). First, parental A2780 cells were treated with various concentrations of VM-26 (20, 40, 80, 150, 300, and 500 ng/ml) for 48 h. Total protein was extracted, electrophoresed on SDS-PAGE, Western blotted, then probed using anti-topoisomerase II antisera. Topoisomerase II levels in parental cells decreased as VM-26 concentrations increased (Fig. 3A). Topoisomerase IIβ decreased to an undetectable level when the VM-26 concentration increased to 300 ng/ml, whereas the topoisomerase IIβ level of the R80-resistant cells decreased slightly as compared to parental cells. The drastic disappearance of topoisomerase IIβ in parental cells at 300 ng/ml of VM-26 might be due to decreased free-form topoisomerase IIβ after VM-26 treatment, together with trapping of the enzyme in the topoisomerase II-DNA complex by VM-26. In contrast, free-form topoisomerase IIβ of the R80-resistant cells was less affected by VM-26. Therefore, the maintenance of topoisomerase IIβ levels in resistant cells might be due to loss of the VM-26 target site in the enzyme. RNA levels of topoisomerases I and II in resistant cells remained fairly constant (Fig. 3B). These results are consistent with the protein data. Decrease of topoisomerase II might not be the major factor leading to VM-26 resistance. The free form of topoisomerase II was not affected by VM-26 in resistant cells implying that topoisomerase II had been mutated, and therefore VM-26 could no longer recognize its target.

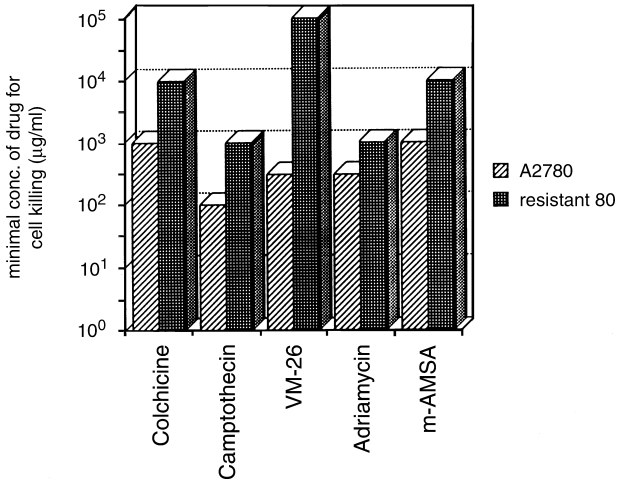

Fig. 1. Cross-resistance test. Colchicine, camptothecin, adriamycin, m-AMSA, and VM-26 were used for the cross-resistance test. Cells were plated in a 24-well plate, and various concentrations of drugs were added into the medium for 72 h at 37 °C. Cells were then stained with methylene blue. R80-resistant cells showed 3-fold tolerance to adriamycin, 10-fold to colchicine, camptothecin, and m-AMSA, and 300-fold to VM-26 when compared to parental A2780 cells.

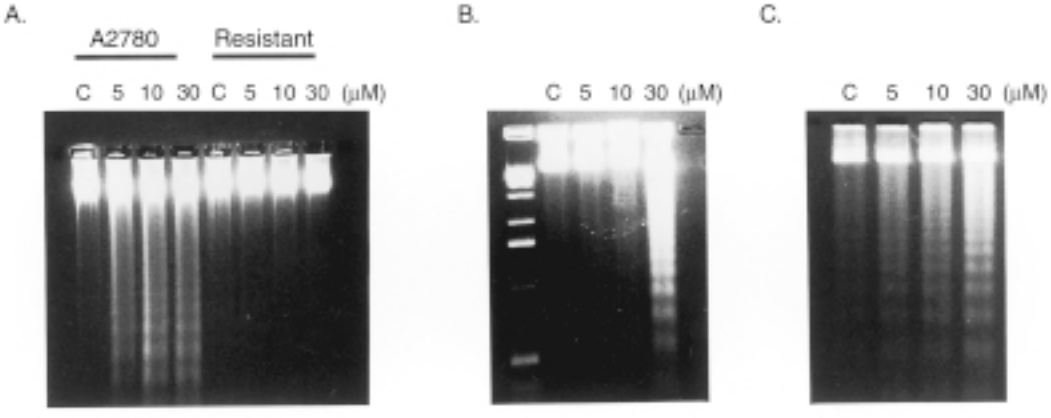

Fig. 2. R80-resistant cells resistant to VM-26-triggered apoptosis. DNA fragmentation of a variety of cells was induced in a dose-dependent manner. Genomic DNA of various concentrations of VM-26 (0, 5, 10, and 30 μM)-treated cells for 24 h was analyzed on a 1.8% agarose gel. Lanes indicated as c were untreated cells, and others are shown as the indicated concentrations. The tested cells were (A) parental A2780 and R80-resistant cells, (B) CHO cells, and (C) NIH3T3 cells.

Topoisomerase II-DNA complex trapping activity less affected by VM-26 treatment in resistant cells

Topoisomerase II can break and rejoin double-stranded DNA; the breaking and rejoining reaction is an equilibrium reaction. The equilibrium favors the rejoining reaction in the absence of VM-26, but favors the breaking reaction in the presence of VM-26 (Zhang et al. 1990). KCl/SDS can precipitate protein, but not DNA except when it is linked to protein. Therefore, the amount of precipitated DNA reflects VM-26 trapping activity. ^{3}H-thymidine-labeled cells were treated with VM-26, and K-SDS precipitation was performed. In parental cells, DNA trapped by VM-26 was approximately 20% at 45.0 μg/ml of the drug (Fig. 4). In the 2 sublines of resistant cells, R40 (40 ng/ml of VM-26) and R80 (80 ng/ml of VM-26), DNA trapped by VM-26 decreased to less than 8% and 5%, respectively (Fig. 4). These results indicate that the ability of VM-26 to cause formation of the topoisomerase II-DNA complex decreased in VM-26-resistant cells. Therefore, it is likely that the VM-26 target site in topoisomerase II of the resistant cells has been mutated, and hence, there is decreased binding of VM-26 to this site.

K-DNA decatenation activity of resistant cells less influenced by VM-26

Catenated kinetoplast DNA was used in the in vitro topoisomerase II decatenation activity assay (Muller et al. 1989). The assay is based on the observation that catenated K-DNA is too large to be electrophoresed into an agarose gel, whereas topoisomerase II-decatenated K-DNA can be. Nuclear extracts from parental and resistant cells were incubated with K-DNA in the absence or presence of VM-26 (5 μg/ml). All the K-DNA remained catenated and stayed at the top of the gel in the reactions that contained no nuclear extracts from parental or resistant cells (Fig. 5, lanes 4 and 7). VM-26 inhibited the topoisomerase II decatenation reaction of the nuclear extract of parental cells (Fig. 5, lanes 2 and 3), but the inhibition was less in resistant cells (Fig. 5, lanes 5 and 6). These results indicate that VM-26 trapped the topoisomerase II-DNA complex in parental cells and led to the absence of free-form topoisomerase II to act on K-DNA. But this trapping effect by VM-26 decreased in the resistant cell nuclear extracts. Topoisomerase II alteration could be the cause of this VM-26 resistance.

DISCUSSION

Multidrug resistance is due to a multidrug transport protein known as p-glycoprotein or p170. This multidrug transporter uses energy from ATP to ex-

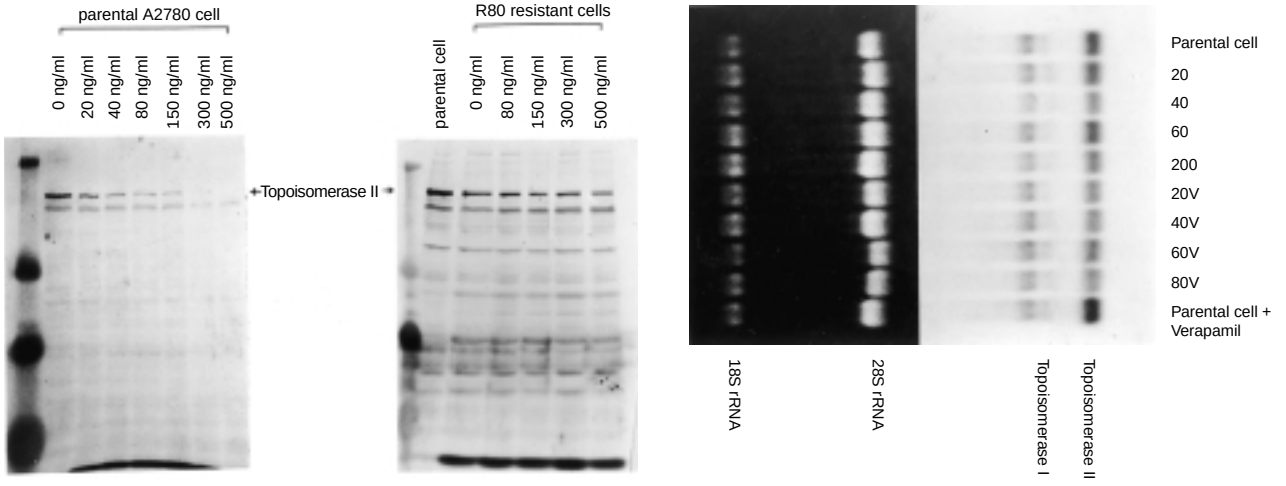

Fig. 3. Topoisomerase protein (A) and RNA (B) levels in VM-26-treated cells. Various concentrations of VM-26 treatment (20, 40, 80, 150, 300, and 500 ng/ml) were used for 48 h in parental cells (left panel) and resistant cells (right panel). Topoisomerase II protein levels decreased as drug concentrations increased in parental cells, but not in R80-resistant cells (A). The mRNAs of topoisomerases I and II show no significant changes in R80-resistant cells compared to those of the parental cells (B). Various concentrations of VM-26 treatment (20, 40, 60, and 200 ng/ml) were used for 48 h in the absence or presence of the anti-MDR drug, verapamil (V).

trude a large variety of drugs from cells (Gottesman and Pastan 1988). The process by this transporter has been identified as one of the mechanisms of drug resistance to chemotherapy in cancer cells. Glutathione S-transferases (GST) are involved in detoxification of drugs in cells (Kramer et al. 1988, Schisselbauer et al. 1989, Parsons et al. 1990). However, the role that GST plays in drug resistance is still not conclusive (Bellamy et al. 1989), therefore, further studies are needed to determine the contribution of GST to drug resistance.

Several series of studies on a human leukemia cell line resistant to VM-26 have been carried out (Beck et al. 1987, Danks et al. 1987, Danks et al. 1988, Wolverton et al. 1989). The T-cell leukemia line resistant to VM-26 has been shown to be associated with an alteration in the activity of DNA topoisomerase II, and to possess cross-resistance to a number of drugs known to stabilize the topoisomerase II-DNA complex, including VP-16, anthracyclines, mitoxantrone, and 4'-(9-acridinylamino) methanesulphon-m-anisidide. This resistance is distinguishable from the MDR associated with Pgp. The effect of VM-26 on stabilization of the topoisomerase II-DNA complex in sensitive cells is more severe than in this resistant cell line. These results have been reproduced in experiments on murine leukemia P388 cells (De Isabella et al. 1990). The alteration in topoisomerase II DNA cleavage activity has been suggested to be a mechanism of VM-26 resistance. Also, KB cells resistant to VP-16 or its derivatives have been cloned, and the resistance has been suggested to be the result of a decrease in topoisomerase II protein, but not MDR associated with P170 (Ferguson et al. 1988, Yu et al. 1997).

We have cloned human ovarian carcinoma A2780 cells that are resistant to VM-26, and we concentrated most of our analyses on the resistant sublines that have developed stable resistance towards 80 ng/ml of VM-26 (R80-resistant cells). The R80 subline showed a cross-resistance of 3 to 10 fold towards colchicin, adriamycin, m-AMSA, and camptothecin, and 300 fold towards VM-26 when compared to parental cells (Fig. 1). Therefore, the resistance of this subline towards VM-26 is more specific. We think that MDR might be a minor factor giving rise to drug resistance in our resistant cells. The doubling time of our resistant cells was slightly less than twice as long as that of the parental cells. The mRNA levels of both topoisomerases I and II stay very constant in parental and resistant cells. Therefore, we think that differences in doubling times, and the reduction of transcriptional activity caused by VM-26 and other drugs cannot fully explain the resistance in our resistant cells or their specificity towards VM-26.

However, when VM-26 concentration reached 300 ng/ml, topoisomerase IIβ protein in parental cells dropped to an almost undetectable level. Yet, topoisomerase IIβ protein levels of the R80-resistant cells fluctuated only slightly, and the largest difference was less than 2 fold when compared to untreated parental cells. Therefore, VM-26 resistance of our resistant cells may be due to the difference between

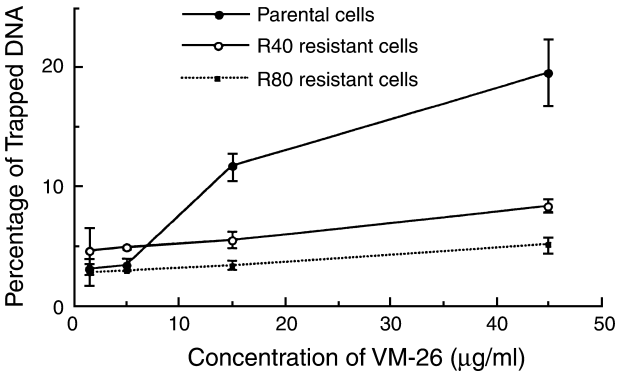

Fig. 4. Topoisomerase II-DNA complex trapping activity by VM-26. Topoisomerase II-DNA complex trapping activity by VM-26 in resistant cells was less than that in parental cells. In vivo K-SDS precipitation shows that the trapped topoisomerase II-DNA complex by VM-26 in resistant cells (R40 and R80) was less than that in parental cells.

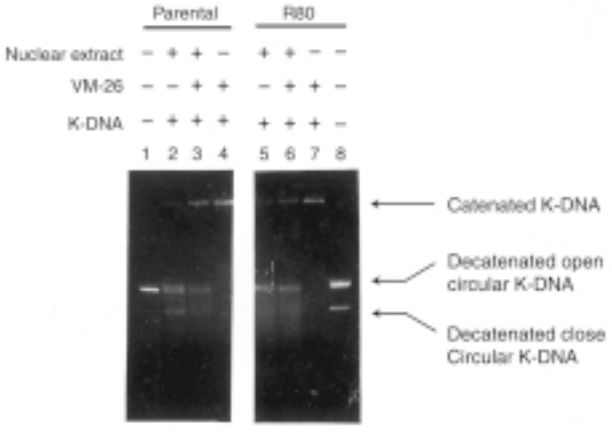

Fig. 5. Topoisomerase II strand-passing activity in parental A2780 and resistant cells. Topoisomerase II strand-passing activity was measured by incubation of 0.2 μg kinetoplast-catenated DNA (K-DNA) with nuclear extract from cells at 30 °C for 10 min in the absence or presence of VM-26 (5 μg/ml). Lane 1, marker, decatenated open circular K-DNA; lane 8, marker, a mixture of decatenated open circular and closed circular K-DNA; lanes 2, 3, reaction with parental extract; lane 3, in the presence of VM-26; lane 4, reaction without nuclear extract; lanes 5, 6, reaction with resistant cell (R80) extract; lane 6, in the presence of VM-26; lane 7, reaction without nuclear extract.

the topoisomerase IIβ protein of the resistant sublines and that of the parental cells. Then, in the K-SDS precipitation experiment, the topoisomerase II-DNA complex trapping activities of the 2 VM-26-resistant sublines were less than that of the parental cells. However, the results of this experiment cannot rule out the possibility that resistance towards VM-26 is due to the decrease in accumulation of VM-26 inside resistant cells. We, therefore, examined topoisomerase II decatenation of K-DNA in vitro. In the presence of 5 μg/ml of VM-26, normal decatenation activity in the nuclear extract from the R80-resistant subline could still be observed, while the nuclear extract from parental cells lost that enzyme activity. It is now apparent that VM-26 resistance of our resistant sublines, at least in the case of the R80-resistant subline, might not come from a decrease in the quantity of the enzyme, but from alteration of the topoisomerase II protein itself. We propose that VM-26 resistance in our resistant subline may come from alteration of the VM-26 target site in the topoisomerase II protein, and this alteration may cause VM-26 to fail to react with topoisomerase II.

Basically, we do not think that 1 single factor will give rise to drug resistance during cancer chemotherapy. Rather, a synergistic effect of multiple factors such as MDR, drug metabolism, and defects in drug-target and cell-killing processes will result in the appearance of drug resistance. We think that defects in drug-target and cell-killing processes play important roles in the development of drug resistance during chemotherapy. A better understanding of all the mechanisms involved will help overcome or prevent resistance to topoisomerase II-targeting drugs.

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A2780 卵巢瘤細胞之 VM-26– 抗藥細胞株之篩選與抗藥機制之探討

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VM-26 是作用在第二型拓撲酵素之抗癌藥物，人類卵巢瘤 A2780 細胞之 VM-26 抗藥株正篩選出來，耐藥性測試結果顯示，抗藥株對 VM-26 耐受性較原母細胞強 300 倍，而對其他的抗癌藥物則只有 3-10 倍的增加。 VM-26 誘發各種細胞之凋亡，但無法在抗藥株細胞有相同效果。顯示盤抗藥株對 VM-26 具專一之耐受性，並非經由多重抗藥性機制。經 VM-26 處理之抗藥株，其第二型拓撲異植酵素訊息 RNA 之表現量並沒有明顯變化，而蛋白質量亦只有 2 倍量之差異，顯示第二型拓撲異構酵素之表現程度與 VM-26 耐藥性形成並沒有明顯關聯性。利用 K-SOS 沈澱法萃取 DNA- 拓撲異構酵素複合物，來定量其 DNA 股交換活性。且利用第二型拓撲異構酵素對 K-DNA 之解套活性，測定 VM-26 對其活性之影響程度。在耐藥株細胞中，其第二型拓撲異構酵素所受 VM-26 影響，遠低於原母細胞株。這些結果暗示，在我們所篩選的 VM-26 耐藥性細胞株中，其抗藥性機制可能源自於第二型拓撲異構酵素中，藥物作用點的突變或改變。

關鍵詞：拓樸異構酵素，抗藥性， VM-26 。

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