Jpn. J. Cancer Res. 82, 1035–1043, September 1991

Characterization of an Etoposide-resistant Human K562 Cell Line, K/eto

Isamu Sugawara,[1,7] Toshiro Iwahashi,[2] Kazuya Okamoto,[3] Yoshikazu Sugimoto,[4] Hisao Ekimoto,[3] Takashi Tsuruo,[5] Tatsuro Ikeuchi[6] and Shigeo Mori[2]

[1]Department of Pathology, Saitama Medical Center, Saitama Medical School, 1981 Kamoda, Kawagoe, Saitama 350, [2]Department of Pathology, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, [3]Oncology Section, Nippon Kayaku Co. Ltd., Shimo, Kita-ku, Tokyo 115, [4]Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 1-37-1 Kita-Ikebukuro, Toshima-ku, Tokyo 170, [5]Institute of Applied Microbiology, The University of Tokyo, 1-1-1 Yayoi Bunkyo-ku, Tokyo 113 and [6]Department of Cytogenetics, Medical Research Institute, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo 113

An etoposide-resistant K562 cell line (K/eto) was obtained by stepwise exposure, in culture, to increasing concentrations of etoposide, without the use of mutagens. This cell line was resistant to etoposide, and slightly resistant to adriamycin, but sensitive to anti-cancer drugs such as camptothecin, vincristine, actinomycin D and so on. P-Glycoprotein, the *mdr*1 gene product, was not detected in this cell line, as assessed by immunocytochemistry, immunoprecipitation and flow cytometry. Overexpression of *mdr*1 mRNA was also not found. Interestingly, expression of 85 kD protein recognized by MRK 20 monoclonal antibody was noted. The level of DNA topoisomerase II protein, detected by antibody staining, decreased concomitantly with a general decrease in DNA topoisomerase II unknotting activity, while DNA topoisomerase I activity was not affected. Cellular accumulation of [^{3}H]etoposide was reduced by 75% in the resistant line compared with parental K562. Karyotype analysis showed that the number of chromosomes in K/eto was 55 and neither a homogeneous staining region nor double-minute chromosomes were detected. These results indicate that this resistance is not due to an altered interaction between the drug and cellular transport machinery, i.e. MDR1, associated with the "classic" multiple drug resistance phenotype, but rather is due to the existence of other mechanism(s) of resistance, decreased transport of the drug and decreased target enzyme, DNA topoisomerase II.

Key words: Etoposide — Multidrug resistance — K562 — 85 kD protein — DNA topoisomerase II

Multidrug resistance (MDR) is a well known phenomenon whereby cells resistant to one anti-cancer drug display cross-resistance to other structurally unrelated anti-cancer drugs.[1] It is necessary to establish a cell line resistant to an anti-cancer drug to dissect the MDR in more detail. Etoposide is commonly used for the treatment of leukemias and lymphomas.[2] Cell lines resistant to etoposide have shown cross-resistance to the *Vinca* alkaloids, as is typical of the MDR phenotype.[3–5] On the other hand, there are reports of cell lines that are cross-resistant to etoposide and sensitive to the *Vinca* alkaloids.[6–8] These apparently contradictory results led us to develop and characterize etoposide-resistant K562 cells. We selected etoposide because etoposide is a derivative of the naturally occurring alkaloid podophyllotoxin and its established target is DNA topoisomerase II, an enzyme that can alter DNA topology by a double-stranded DNA breaking-resealing reaction.[2] We examined etoposide-resistant K562 cells in terms of karyotyping, DNA topoisomerases, the 50% growth-inhibitory concentrations (IC_{50}), P-glycoprotein, 85 kD protein recognized by MRK 20 monoclonal antibody and cellular accumulation of [^{3}H]etoposide. We report here the isolation and characterization of an etoposide-resistant K562 that may express MDR different from "classic" MDR.

MATERIALS AND METHODS

Cell lines Human myelogenous leukemia K562 cells were obtained from one of the present authors and were maintained as described previously.[9,10] An etoposide-resistant cell line (K/eto) was obtained by sequential selection, by using increasing concentrations of etoposide, without the use of mutagens. Selection was begun by exposing a culture of K562 cells to 100 ng/ml etoposide, and after one month this was increased to 200 ng/ml. Thereafter, this was increased to 800 ng/ml as a final concentration. Under these conditions and subsequent selection conditions a clonal line was obtained from several clones based on the most rapid rate of proliferation in the presence of the drug. The selection of a stable clone, K/eto, took one year from the beginning of

[7] To whom correspondence should be addressed.

exposure to 100 ng/ml etoposide. A colchicine-selected multidrug-resistant derivative, KB-8-5, has been reported.[11, 12] An adriamycin-selected multidrug-resistant derivative, K562/ADM, has also been described.[9]

Drugs Etoposide, bleomycin and cisplatin were gifts from Nippon Kayaku Co., Ltd., Tokyo. Adriamycin was kindly provided by Kyowa Hakko Co., Ltd., Tokyo. Vincristine, colchicine, actinomycin D and methotrexate were purchased from Sigma Chemical Co., St. Louis, MO. Camptothecin was a gift from Yakult Co., Ltd., Tokyo. [^{3}H]Etoposide was purchased from Moravek Biochemicals (Brea, CA).

Antibodies MRK 16 and MRK 20 monoclonal antibodies (MAb) were prepared by one of the present authors.[13, 14] Rabbit serum containing antibodies to DNA topoisomerase II was a generous gift of Dr. L. Liu, Johns Hopkins University, Baltimore, MD.[15] The MAb to DNA topoisomerase I was prepared and characterized by Dr. A. Kikuchi, Mitsubishi Kasei Institute of Life Sciences, Tokyo.

Drug toxicity Cells (3×10^5) were cultured at 37°C for 5 h in 24-well culture wells containing 1.5 ml of RPMI 1640 with 10% fetal calf serum. Then they were treated with graded concentrations of etoposide or other anti-cancer drugs (0.001–10 μM). The cells were cultured in the presence of the drug and counted in a hemocytometer three days after drug treatment. Three wells were used for each drug concentration. The cytotoxic activity of the drug was measured by determining the IC_{50}, which was obtained by plotting the logarithm of the drug concentration versus the growth rate (percentage of control) of the treated cells. The initial cell number was subtracted in the calculation.[9]

Determination of cellular drug content K/eto and K562 cells (3×10^6/ml each) were incubated in the presence of 10 μM [^{3}H]etoposide at 37°C for 20, 40, and 60 min. The cells were washed three times with ice-cold PBS. The radioactivity of cell precipitates treated with 4 N NaOH and 5 N HCl at room temperature was determined in a liquid scintillation counter.[16]

In order to examine outward transport of [^{3}H]etoposide, K/eto and K562 cells were incubated in the presence of 10 μM [^{3}H]etoposide at 37°C for 60 min. The cells were washed three times with ice-cold PBS. The cell precipitates in RPMI 1640 were incubated at 37°C for 20, 40, and 60 min. Then the cells were washed three times with ice-cold PBS. The radioactivity of the cell precipitates was determined in a liquid scintillation counter.

Cytogenetic studies For chromosome analysis, exponentially growing cells were treated with colcemid (0.04 μg/ml) for 2 h. The cells were harvested, treated with 0.075 M KCl for 20 min at 37°C, fixed in methanol-acetic acid (3:1) solution, and spread on dry slides. Quinacrine staining was used for detailed karyotype analysis.[17]

Immunocytochemistry Immunocytochemistry was carried out as described previously.[18, 19] MAbs MRK 16 and MRK 20 were used as the first antibodies.

Immunoelectron microscopy Immunoelectron microscopy was performed using MAb MRK 16 and K/eto cells as described previously.[18]

Flow cytometry Flow cytometry was carried out using MAbs MRK 16 and MRK 20 as described previously.[19]

Western blotting To perform Western blotting to demonstrate DNA topoisomerases I and II, nuclei from K562 and K/eto cells were first isolated as described elsewhere.[20] Thereafter, the polyethylene glycol (PEG) supernatant was prepared from the nuclei from 2.5 g of fresh K562 and K/eto cells. The nuclei were resuspended in 5 ml of nuclei wash buffer, and ethylenediaminetetraacetic acid (EDTA) was added to a final concentration of 4 mM. After 15 min at 0°C, the nuclei were lysed by the slow addition, with stirring, of 5 ml of 2 M NaCl, 50 mM Tris-HCl, pH 7.5, 10 mM mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride (PMSF). After another 15 min at 0°C, 5 ml of 8% (w/v) PEG, 1 M NaCl, 50 mM Tris-HCl, pH 7.5, 10 mM mercaptoethanol, and 1 mM PMSF was slowly added with constant stirring. This solution was incubated for 40 min at 0°C with occasional stirring and then centrifuged at 12,000g for 30 min. The supernatants were used for Western blotting. Western blotting was carried out using antibodies against DNA topoisomerases I and II as described previously. Western blotting using solubilized proteins from K562 and K/eto cells was also performed to demonstrate the presence of 85 kD protein recognized by MRK 20.

Immunoprecipitation Immunoprecipitation was done to examine the presence of P-glycoprotein as described previously.[21, 22]

RNA expression cDNA was prepared by reverse transcription of 2 μg of total RNA using 200 ng of random hexanucleotide primer (Takara Shuzo Co., Kyoto) and 200 units of MMuLV reverse transcriptase (Bethesda Research Labs.) under the conditions recommended by the supplier. Total RNA was prepared as described previously.[23] Aliquots of cDNA equivalent to 0.2 μg of total RNA were used for enzymatic amplification by polymerase chain reaction (PCR) using 0.6 unit of *Taq* DNA polymerase (Perkin Elmer Cetus) in the presence of 1 μM of *mdr*1-specific primers (positions 2314–2334 and 2889–2909 in the cDNA sequence) and β_2-microglobulin-specific primers (1556–1575 and 3558–3577 in the genomic sequence).[24] Thirty cycles of PCR were carried out in 50 μl volume using a thermal cycler. Each cycle included 1 min of denaturation at 94°C, followed by 5 min of primer annealing and extension at 65°C. Because the efficiency of the amplification process is greatly affected by a number of variables including yield and quality of cDNA preparations, the overall efficiency of individual

PCR reactions was first calculated by measuring the amount of the coamplified 261-bp β_2-microglobulin-specific band, then 2 μl and 4 μl of 1/5 diluted reaction mixtures were analyzed by electrophoresis on a 1.2% agarose gel, and the amount of the β_2-microglobulin-specific DNA was estimated in terms of ethidium bromide-mediated fluorescence. A 5 to 20 μl aliquot of each reaction mixture was then analyzed by electrophoresis on a 1.2% agarose gel, followed by ethidium bromide staining, and Southern hybridization with 5A probe for *mdr*1 RNA.[25,26]

DNA topoisomerase II assay The standard DNA topoisomerase II reaction mixture (20 μl) contained 50 m*M* Tris-HCl, pH 7.9, 120 m*M* KCl, 10 m*M* $MgCl_2$, 0.5 m*M* dithiothreitol, 0.5 m*M* EDTA, 30 μg/ml of bovine serum albumin, 0.5 m*M* ATP, 20 μg/ml of DNA. For the unknotting reactions, knotted P_4 DNA was used in the standard reaction.[15]

RESULTS

***In vitro* drug cytotoxicity** The 72-h IC_{50} values of several drugs for the parent drug-sensitive (K562) and an etoposide-resistant (K/eto) cell line are shown in Table I. K/eto showed a high degree of resistance to etoposide (17.1-fold). The cells were moderately resistant to adriamycin (7.6-fold). The cells were sensitive to vincristine, colchicine, cisplatin, bleomycin, actinomycin D, methotrexate and camptothecin.

Characterization of K/eto The K/eto line was cloned by limiting dilution and retained its resistance to etoposide for one month in the absence of the drug. The doubling time of K/eto was ca. 40 h, while that of K562 was ca. 24 h. The K/eto cells did not possess P-glycoprotein as evaluated by immunocytochemistry (Fig. 1), flow cytometry (Fig. 2) and immunoprecipitation (data not shown). However, K/eto did possess 85 kD protein recognized by MRK 20 (Figs. 1 and 2). Fig. 3 shows the result of Western blotting when MRK 20 MAb was used as the first antibody. Immunoelectron microscopy has

Table I. Sensitivity of K562/eto to Various Drugs

Drug	IC_{50} (μM)		
	K562	K562/eto	
Etoposide	0.14	2.4	(17.1)[a]
Adriamycin	0.011	0.08	(7.6)
Vincristine	<0.001	<0.001	
Colchicine	0.0028	0.0038	(1.4)
Cisplatin	0.98	0.55	(0.6)
Bleomycin	0.7	0.4	(0.6)
Actinomycin D	0.003	0.003	(1.0)
Methotrexate	0.011	0.001	(0.1)
Camptothecin	0.0025	0.0027	(1.1)

a) Relative resistance.

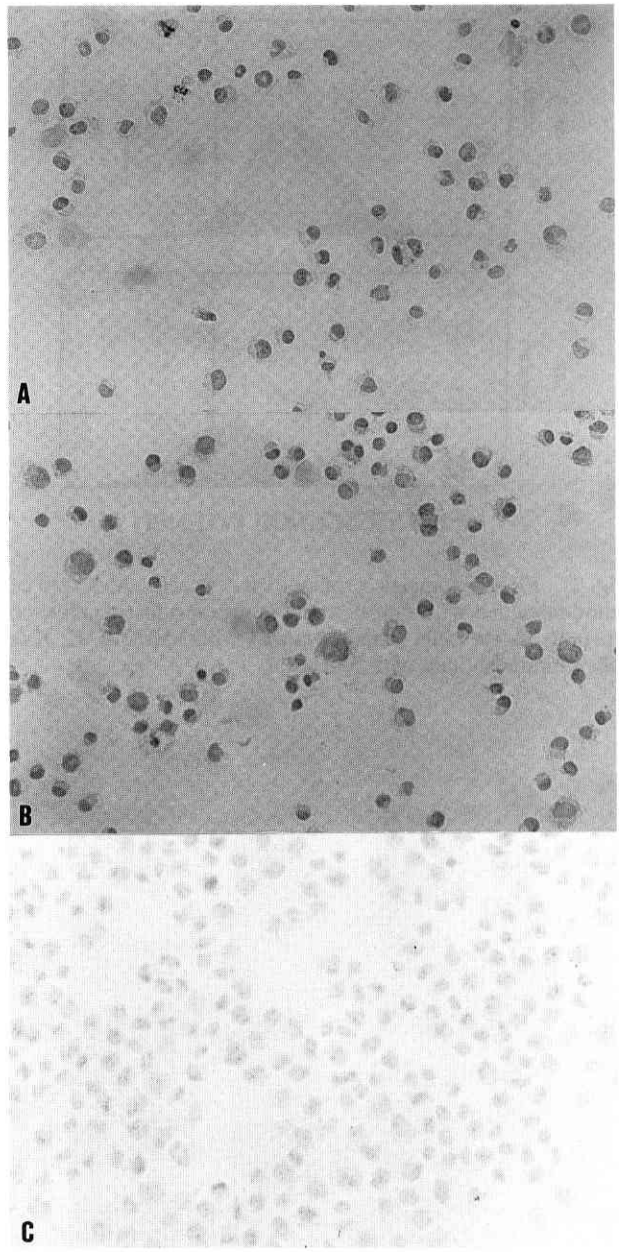

Fig. 1. Immunocytochemistry of K/eto and K562. The ABC-PO method was used. A, K/eto cells immunostained with MRK 16. ×400. B, K/eto cells immunostained with MRK 20. ×400. C. K/eto cells immunostained with non-immune mouse serum. ×400.

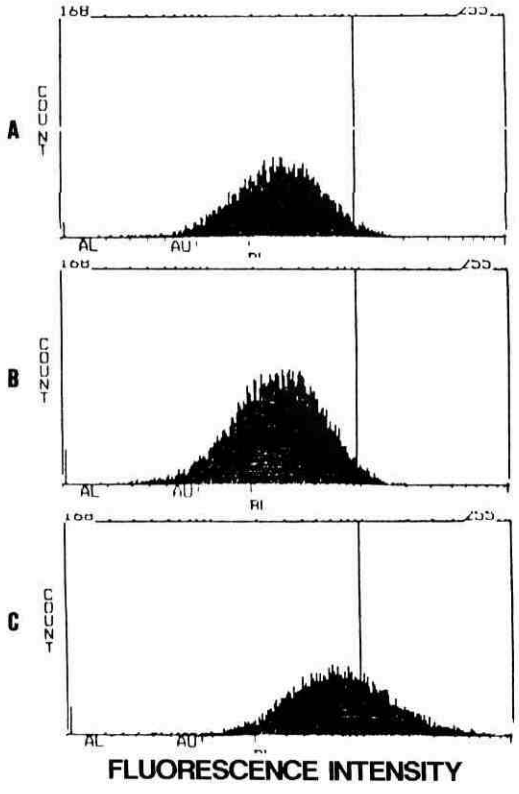

Fig. 2. Flow cytometric profiles of K/eto cells. A, K/eto cells immunostained with non-immune mouse serum (negative control). B, K/eto cells immunostained with MRK16. C, K/eto cells immunostained with MRK 20.

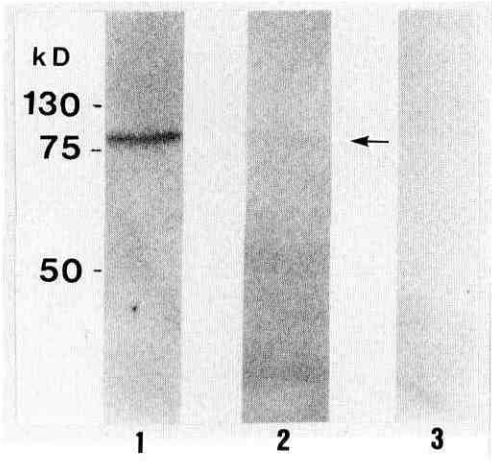

Fig. 3. Western blot of solubilized proteins from K/eto, K562 and K562/ADM cells. Solubilized proteins from K562/ADM (lane 1), K/eto (lane 2) and K562 (lane 3) immunostained with MRK 20.

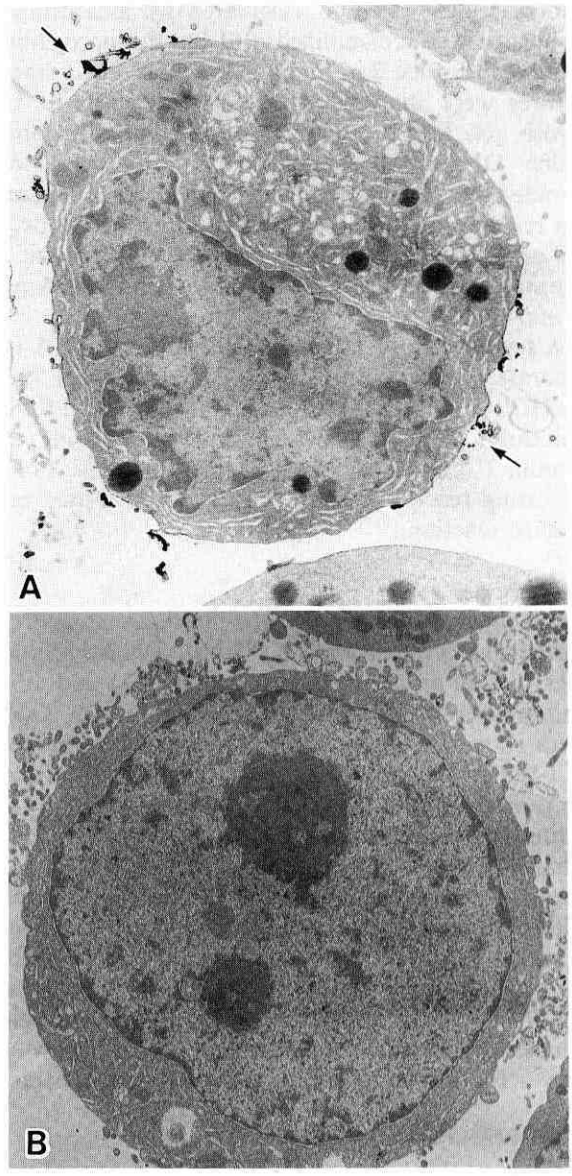

Fig. 4. Immunoelectron micrograph of K/eto immunostained with MRK 20. A, MRK 20. B, non-immune mouse serum instead of the first antibody. The 85 kD protein is distributed heterogeneously in the cell membrane of the K/eto cell (arrow).

revealed that this 85 kD protein was distributed heterogeneously in the cell membranes of the K/eto cells (Fig. 4).

***mdr*1 gene analysis of K/eto** The data in Fig. 5 show that K/eto does not overexpress the *mdr*1 gene as evaluated by enzymatic amplification after reverse transcription of *mdr*1 mRNA into the cDNA.

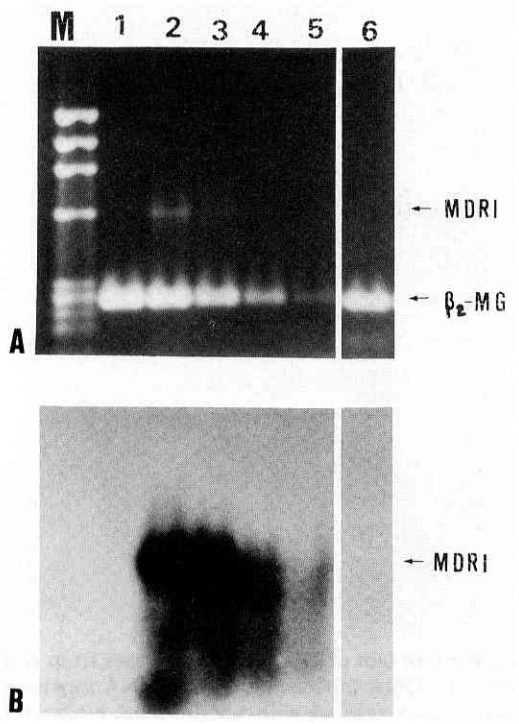

Fig. 5. *mdr*1 gene analysis of K/eto cells. A, Detection of *mdr*1 mRNA by enzymatic amplification. After electrophoresis the product was detected by ethidium bromide staining. M, *Hae*III-digested ox 174 DNA. lane 1, KB 3-1. 2, KB 8-5. 3, 5-fold-diluted KB 8-5. 4, 25-fold-diluted KB 8-5. 5, 125-fold-diluted KB 8-5. 6, K/eto. B, Southern blot hybridization of PCR products.

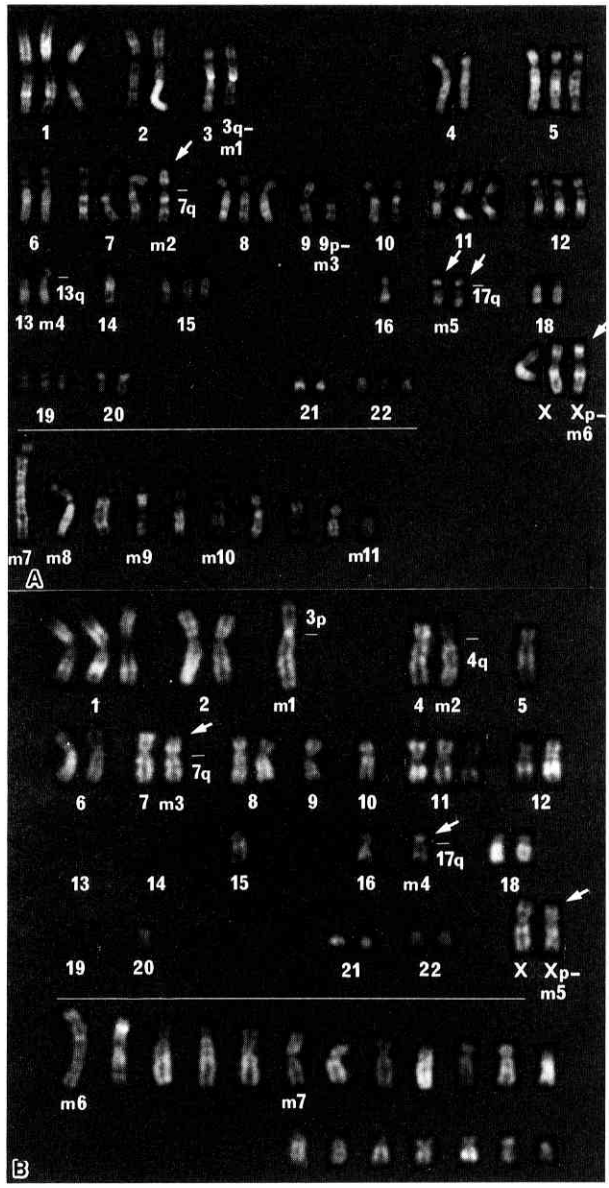

Fig. 6. Cytogenetic analysis of K/eto. A, A Q-banded karyotype (65 chromosomes) of the cell line K562. Structurally abnormal marker chromosomes whose origins were not identifiable are arranged below the white line. The marker chromosomes found in more than 40% of the cells analyzed are numbered as m1-m11. The arrows indicate the common markers between K562 and K/eto cells. B, A Q-banded karyotype (55 chromosomes) of the K/eto line, a subline derived from the K562 cell line.

Cytogenetic analysis The number of chromosomes in K562 was 65, while that in K/eto was 55. Neither a homogeneous staining region nor double-minute chromosomes were detected in K/eto (Fig. 6).

Cellular pharmacology of [^{3}H]etoposide In the presence of 10 μM [^{3}H]etoposide, the accumulation of the drug in K562 reached a plateau after incubation for 10 min at 37°C, and approximately 18 pmol of etoposide was incorporated into 10^6 cells (Fig. 7). Incorporation of [^{3}H]-etoposide into K/eto cells was approximately 1/5 of that found in K562 cells.

Outward transport of the drug was examined at 37°C in the growth medium without etoposide. At 37°C a rapid efflux of the drug occurred during incubation for 10 min. No clear difference in drug efflux was detected between K562 cells and K/eto cells (Fig. 7).

Detection of DNA topoisomerases I and II by antibody staining The PEG supernatants containing nuclear proteins were subjected to SDS-polyacrylamide gel electrophoresis in 7.5% acrylamide. Approximately 100 μg of protein was applied per lane for K562 and K/eto cells.

Fig. 8A shows that 97 kD bands corresponding to DNA topoisomerase I were recognized in K562 and K/eto cells and that there was no difference in staining intensity between them.

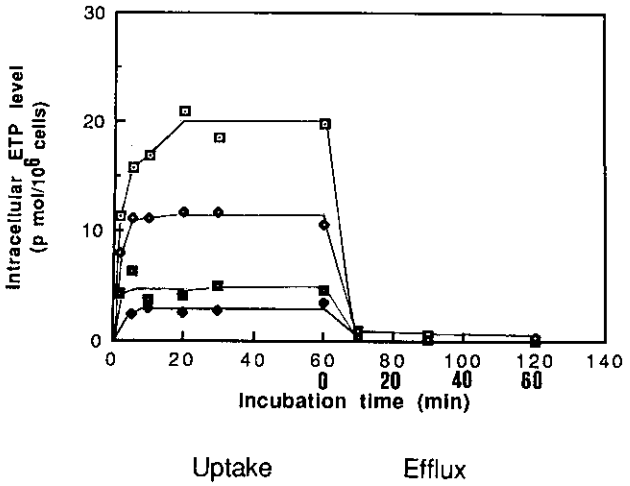

Fig. 7. Uptake and efflux of [^{3}H]etoposide. K/eto and K562 cells (3×10^6/ml) were incubated in the presence of 10 μM [^{3}H]-etoposide at 37°C for 20, 40 and 60 min. The cells were washed with ice-cold PBS. For outward transport of [^{3}H]etoposide, K/eto and K562 cells were incubated in the presence of 10 μM [^{3}H]etoposide at 37°C for 60 min. Thereafter, the cell precipitates were incubated at 37°C for 20, 40 and 60 min. Then the cells were washed with ice-cold PBS. The radioactivity of the cell precipitates was determined in a liquid scintillation counter. ⊡; K562, ◆; K/A, ■; K/eto.

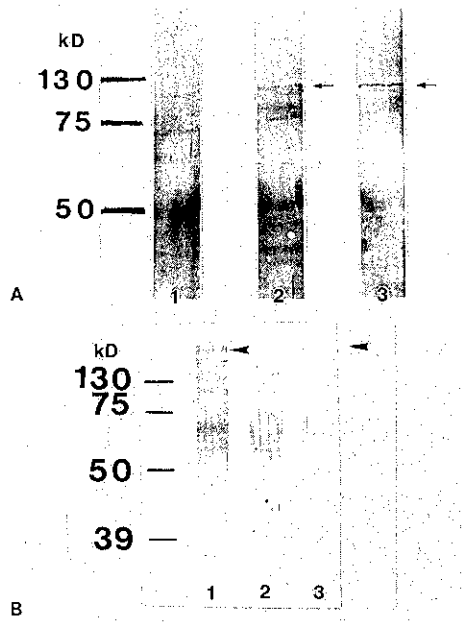

Fig. 8. Western blot of DNA topoisomerases from K/eto and K562 cells. A, DNA topoisomerase I. 1, DNA topoisomerase I from K/eto cells reacted with non-immune mouse serum. 2, DNA topoisomerase I from K/eto cells reacted with anti-DNA topoisomerase antibody. 3, DNA topoisomerase I from K562 reacted with anti-DNA topoisomerase I antibody. B, DNA topoisomerase II. 1, DNA topoisomerase II from K562 reacted with anti-DNA topoisomerase II antibody. 2, DNA topoisomerase II from K/eto reacted with non-immune rabbit serum. 3, DNA topoisomerase II from K/eto reacted with anti-DNA topoisomerase II antibody.

DNA topoisomerase II bands (180 kD) were recognized in K562 and K/eto cells and there was a decrease of DNA topoisomerase II in K/eto cells, although the same amount of protein was loaded onto each lane for a given antibody staining (Fig. 8B).

Assay of DNA topoisomerase II activity Activity of DNA topoisomerase II was assayed in terms of the ability to unknot P_4 phage knotted DNA. The samples were run on 1% agarose gels and visualized by ethidium bromide staining and UV irradiation. DNA topoisomerase II activity was recognized by the conversion of the smear of knotted DNA to a band of unknotted DNA at the top of the gel. Although PEG in DNA topoisomerase II-containing reaction mixture inhibited DNA topoisomerase II activity (lanes 2, 3, 7 and 8 in Fig. 9), the DNA topoisomerase II activity in serially diluted samples derived from the K/eto cells was generally about one-third of that of K562 cells (Fig. 9).

DISCUSSION

We have shown that our etoposide-resistant K562 line, K/eto, does not possess P-glycoprotein and has decreased DNA topoisomerase II activity. Possible mechanisms of etoposide or teniposide resistance may be as follows; (a) reduced level of DNA topoisomerase II, (b) altered DNA topoisomerase II with a reduced drug sensitivity, (c) reduced cellular accumulation of etoposide or teniposide, (d) reduction of DNA topoisomerase II because of altered nuclear factor(s) associated with the enzyme and (e) the existence of membrane protein(s) other than P-glycoprotein responsible for outward transport of the drug.[7,27-29] Our results raise several points that warrant discussion. First, K/eto cell line is moderately resistant to adriamycin but sensitive to other drugs examined. Although several etoposide-resistant cell lines have been reported,[4,7,16] they are resistant to vincristine. Our cell line is sensitive to vincristine. K562 used in this study was not resistant to adriamycin or vincristine, although K562 cells are *mdr*1-positive. K/eto was sensitive to methotrexate as reported elsewhere.[16] These findings suggest that there is an altered interaction between the drug and its cellular target(s) such as membrane protein(s).

Second, cellular pharmacology has revealed that there is a reduced accumulation of [^{3}H]etoposide in K/eto,

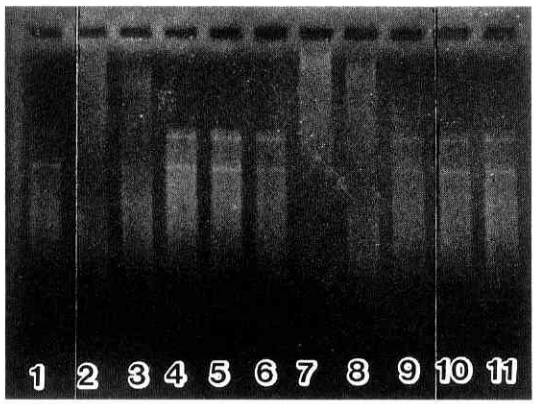

Fig. 9. Assay of DNA topoisomerase II activity. 1, Knotted P_4 DNA. 2, Knotted P DNA in the presence of 2-fold-diluted PEG supernatant from K562. 3, Knotted P_4 DNA in the presence of 4-fold-diluted PEG supernatant from K562. 4, 8-fold-diluted PEG supernatant from K562. 5, 16-fold-diluted PEG supernatant from K562. 6, 32-fold-diluted PEG supernatant from K562. 7, PEG supernatant from K/eto. 8, 2-fold-diluted PEG supernatant from K/eto. 9, 4-fold-diluted PEG supernatant from K/eto. 10, 16-fold-diluted PEG supernatant from K/eto. 11, 32-fold-diluted PEG supernatant from K/eto.

compared with K562. No difference has been found in retention of etoposide between K/eto and K562 cells, although it was reported that there was a difference in retention of etoposide between CEM and CEM/VLB_{100} cells.[3] This may reflect alteration of the membranes in K/eto. K/eto cells may have another outward transport system of etoposide, because K/eto cells do not possess P-glycoprotein.

Third, our cell line did not possess P-glycoprotein as evaluated by immunocytochemistry and immunoblotting. Furthermore, there was no overexpression of *mdr*1 mRNA in our cell line. Our findings are consistent with the reports by Ferguson *et al.*[16] and Beck *et al.*[30] There may be a protein (130 kD) other than P-glycoprotein in various cell lines,[31-37] and K/eto cells may possess this protein.

Recently it was reported that transport proteins other than P-glycoprotein are present in a daunorubicin-resistant variant of K562 and adriamycin-resistant H69.[38,39] Our cell line possesses an 85 kD protein recognized by MRK 20 secreted from a clone obtained by immunizing K562/ADM into BALB/c mice.[14,19] This protein(s) may be responsible for MDR although its role remains to be clarified.

Finally, our cell line has lower DNA topoisomerase II activity as well as DNA topoisomerase II content. However, this cell line was sensitive to camptothecin, a DNA topoisomerase I inhibitor, and DNA topoisomerase I content was not reduced. This finding is in contrast with that by Ferguson *et al.* that the levels of DNA topoisomerase I activity and enzyme increased compensatorily in the etoposide-resistant KB cell lines.[16] Further study will be required to reconcile these results. Very recently Matsuo *et al.* have reported that teniposide-resistant human cancer KB cell lines show reduced drug accumulation and DNA topoisomerase II.[40] Our cell line is similar to theirs in terms of drug accumulation and DNA topoisomerase II activity.

In summary, there are two possibilities for acquisition of resistance to etoposide in our cell line. The action of etoposide in causing DNA topoisomerase II-dependent double-stranded DNA breaks is well established.[2] Since there is low DNA topoisomerase II activity and content in the cell line, etoposide may be a DNA topoisomerase II-interactive drug. There also exists a very important mechanism of resistance to etoposide other than the presence of P-glycoprotein at the cell surface level, and this may have been responsible for decreased drug accumulation.

(Received March 16, 1991/Accepted June 10, 1991)

REFERENCES

1) Pastan, I. and Gottesman, M. Multiple-drug resistance in human cancer. *N. Engl. J. Med.*, **316,** 1388–1393 (1987).
2) Long, B. H. and Stringfellow, D. A. Inhibitors of topoisomerase II: structure-activity relationships and mechanism of action of podophyllin congeners. *Adv. Enzyme Regul.*, **27,** 223–256 (1988).
3) Danks, M. K., Yalowich, J. C. and Beck, W. T. Atypical multiple drug resistance in a human leukemic cell line selected for resistance to teniposide (VM-26). *Cancer Res.*, **47,** 1297–1301 (1987).
4) Hill, B. T. and Whelan, R. D. H. Establishment of vincristine-resistant and vindesine-resistant lines of murine lymphoblasts *in vitro* and characterization of their patterns of cross-resistance and drug sensitivities. *Cancer Chemother. Pharmacol.*, **8,** 163–169 (1982).
5) Glisson, B., Gupta, R., Smallwood-Kentro, S. and Ross, W. Characterization of acquired epipodophyllotoxin resistance in a Chinese hamster ovary cell line: loss of drug-stimulated DNA cleavage activity. *Cancer Res.*, **46,** 1934–1938 (1986).
6) Johnson, R. K. and Howard, R. S. Development and cross-resistance characteristics of a subline of P388 leukemia resistant to 4′-(9-acridinylamino)methanesulfonyl-*m*-anisidine. *Eur. J. Clin. Oncol.*, **18,** 479–487 (1982).

7) Gupta, R. S. Genetic, biochemical, and cross-resistance studies with mutants of Chinese hamster ovary cells resistant to the anticancer drugs, VM-26 and VP-16-213. *Cancer Res.*, **43**, 1568–1574 (1983).
8) Odaimi, M., Andersson, B. S., McCredie, K. B. and Beran, M. Drug sensitivity and cross-resistance of the 4′-(9-acridinylamino)methanesulfonyl-*m*-anisidine-resistant subline of HL-60 human leukemia. *Cancer Res.*, **46**, 3330–3333 (1986).
9) Tsuruo, T., Iida-Saito, H., Kawabata, H., Oh-hara, T., Hamada, H. and Utakoji, T. Characteristics of resistance to adriamycin in human myelogenous leukemia K562 resistant to adriamycin and in isolated clones. *Jpn. J. Cancer Res.*, 77, 682–692 (1986).
10) Lozzio, C. B. and Lozzio, B. B. Human chronic myelogenous leukemia cell line with positive Philadelphia chromosomes. *Blood*, **45**, 321–328 (1975).
11) Akiyama, S., Fojo, A., Hanover, J. A., Pastan, I. and Gottesman, M. M. Isolation and genetic characterization of human KB cell lines resistant to multiple drugs. *Somatic Cell Mol. Genet.*, **11**, 117–126 (1985).
12) Shen, D. W., Fojo, A., Chin, J. E., Roninson, I. B., Richert, N., Pastan, I. and Gottesman, M. M. Human multidrug resistant cell lines: increased *mdr*1 expression can precede gene amplification. *Science*, **232**, 643–645 (1986).
13) Hamada, H. and Tsuruo, T. Functional role for the 170- to 180-kDa glycoprotein specific to drug-resistant tumor cells as revealed by monoclonal antibodies. *Proc. Natl. Acad. Sci. USA*, **83**, 7785–7789 (1986).
14) Hamada, H., Okochi, E., Watanabe, M., Oh-hara, T., Sugimoto, Y., Kawabata, H. and Tsuruo, T. Mr. 85,000 membrane protein specifically expressed in adriamycin-resistant human tumor cells. *Cancer Res.*, **48**, 7082–7087 (1988).
15) Halligan, B. D., Edwards, K. A. G. and Liu, L. F. Purification and characterization of a type II DNA topoisomerase from bovine calf thymus. *J. Biol. Chem.*, **260**, 2475–2482G (1985).
16) Ferguson, P. J., Fisher, M. H., Stephenson, J., Li, D-H., Zhou, B-S. and Cheng, Y-C. Combined modalities of resistance in etoposide-resistant human KB cell lines. *Cancer Res.*, **48**, 5956–5964 (1988).
17) Fukuhara, S. and Rowley, J. D. Chromosome 14 translocations in non-Burkitt lymphomas. *Int. J. Cancer*, **22**, 14–21 (1978).
18) Sugawara, I., Kataoka, I., Morishita, Y., Hamada, H., Tsuruo, T., Itoyama, S. and Mori, S. Tissue distribution of P-glycoprotein encoded by a multidrug-resistant gene as revealed by a monoclonal antibody, MRK 16. *Cancer Res.*, **48**, 1926–1929 (1988).
19) Sugawara, I., Ohkochi, E., Hamada, H., Tsuruo, T. and Mori, S. Cellular and tissue distribution of MRK20 murine monoclonal antibody-defined 85-kDa protein in adriamycin-resistant cancer cell lines. *Jpn. J. Cancer Res.*, **79**, 1101–1110 (1988).
20) Miller, K. G., Liu, L. F. and Englund, P. T. A homogeneous type II DNA topoisomerase from HeLa cell nuclei. *J. Biol. Chem.*, **256**, 9334–9339 (1981).
21) Hamada, H. and Tsuruo, T. Growth inhibition of multidrug-resistant cells by monoclonal antibodies against P-glycoprotein. *In* "Molecular and Cellular Biology of Multidrug Resistance in Tumor Cells," ed. I. Roninson (1991). Plenum Press, New York. (in press)
22) Sugawara, I., Nakahama, M., Hamada, H., Tsuruo, T. and Mori, S. Apparent stronger expression in the human adrenal cortex than in the human adrenal medulla of Mr 170,000–180,000 P-glycoprotein. *Cancer Res.*, **48**, 4611–4614 (1988).
23) Maniatis, T., Fritsch, E. F. and Sambrook, J. "Molecular Cloning: A Laboratory Manual" (1982). Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.
24) Gussow, D., Rein, R., Ginjaar, I., Hochsteinbach, F., Seemann, G., Kottman, A. and Ploegh, H. L. The human β_2-microglobulin gene primary structure and definition of the transcription unit. *J. Immunol.*, **139**, 3132–3138 (1987).
25) Ueda, K., Cardarelli, C., Gottesman, M. M. and Pastan, I. Expression of a full-length cDNA for the human *mdr*1 (P-glycoprotein) gene confers multidrug-resistance. *Proc. Natl. Acad. Sci. USA*, **84**, 3004–3008 (1987).
26) Noonan, K. E. and Roninson, I. B. mRNA phenotyping by enzymatic amplification of randomly primed cDNA. *Nucleic Acids Res.*, **16**, 10366 (1988).
27) Deffie, A. M., Batra, J. K. and Goldenberg, G. J. Direct correlation between DNA topoisomerase II activity and cytotoxicity in Adriamycin-sensitive and -resistant P388 leukemia cell lines. *Cancer Res.*, **49**, 58–62 (1989).
28) Danks, M. K., Schmidt, C. A., Cirtain, M. C., Suttle, D. P. and Beck, W. T. Altered catalytic activity of and DNA cleavage by DNA topoisomerase II from human leukemia cells selected for resistance to VM-26. *Biochemistry*, **27**, 8861–8869 (1988).
29) Sullivan, D. M., Latham, M. D., Row, T. C. and Ross, W. E. Purification and characterization of an altered topoisomerase II from a drug-resistant Chinese hamster ovary cell line. *Biochemistry*, **28**, 5680–5687 (1989).
30) Beck, W. T., Cirtain, M. C., Danks, M. K., Felsted, R. L., Safa, A. R., Wolverton, J. S., Suttle, D. P. and Trent, J. M. Pharmacological, molecular, and cytogenetic analysis of "atypical" multidrug-resistant human leukemic cells. *Cancer Res.*, **47**, 5455–5460 (1987).
31) Kartner, N., Evernden-Porelle, D., Bradley, G. and Ling, V. Detection of P-glycoprotein in multidrug-resistant cell lines by monoclonal antibodies. *Nature*, **316**, 820–823 (1985).
32) Center, M. S. Mechanisms regulating cell resistance to Adriamycin. Evidence that drug accumulation in resistant cells is modulated by phosphorylation of a plasma membrane glycoprotein. *Biochem. Pharmacol.*, **34**, 1471–1476 (1985).
33) Peterson, R. H. F., Meyers, M. B., Spengler, B. A. and

Biedler, J. L. Alteration of plasma membrane glycopeptides and gangliosides of Chinese hamster cells accompanying development of resistance to daunorubicin and vincristine. *Cancer Res.*, **43**, 222–228 (1983).

34) Beck, W. T., Mueller, T. J. and Tanzer, L. R. Altered surface membrane glycoproteins in *Vinca* alkaloid-resistant human leukemic lymphoblasts. *Cancer Res.*, **39**, 2070–2076 (1979).

35) Riordan, J. R. and Ling, V. Purification of P-glycoprotein from plasma membranes vesicles of Chinese hamster ovary cell mutants with reduced colchicine permeability. *J. Biol. Chem.*, **254**, 12701–12705 (1979).

36) Shen, D-W., Cardarelli, C., Hwang, J., Cornell, M., Richert, N., Ishii, S., Pastan, I. and Gottesman, M. M. Multiple drug-reistant human KB carcinoma cells independently selected for high-level resistance to colchicine, adriamycin, or vinblastine show changes in expression of specific proteins. *J. Biol. Chem.*, **261**, 7762–7770 (1986).

37) Danks, M. K., Metzger, D. W., Ashmun, R. A. and Beck, W. T. Monoclonal antibodies to glycoproteins of *Vinca* alkaloid-resistant human leukemic cells. *Cancer Res.*, **45**, 3220–3224 (1985).

38) Yanovich, S., Hall, R. E. and Gewirtz, D. A. Characterization of a K562 multidrug-resistant cell line. *Cancer Res.*, **49**, 4499–4503 (1989).

39) Mirski, S. E. L. and Cole, S. P. C. Antigens associated with multidrug resistance in H69AR, a small cell lung cancer cell line. *Cancer Res.*, **49**, 5719–5724 (1989).

40) Matsuo, K., Kohno, K., Takano, H., Sato, S., Kiue, A. and Kuwano, M. Reduction of drug accumulation and DNA topoisomerase II activity in acquired Teniposide-resistant human KB cell lines. *Cancer Res.*, **50**, 5819–5824 (1990).