Jpn. J. Cancer Res. 88, 1218–1223, December 1997

Enhanced Topoisomerase I Activity and Increased Topoisomerase IIα Content in Cisplatin-resistant Cancer Cell Lines

Yukihisa Minagawa,[1] Junzo Kigawa, Takashi Irie, Yasunobu Kanamori, Hiroaki Itamochi, Xiusi Cheng and Naoki Terakawa

Department of Obstetrics and Gynecology, Tottori University School of Medicine, 36-1 Nishimachi, Yonago 683

Although the combined effects of cisplatin (CDDP) and DNA topoisomerase (Topo) inhibitors have been described in recent literature, little is known about the combined effects and their biological basis in CDDP-resistant cells. The aim of the present study was to elucidate the combined effect of CDDP and Topo inhibitors on CDDP-resistant cells as well as to investigate the biological factors involved in the sensitivity to these anti-cancer agents. We found synergistic actions between CDDP and SN-38 (a Topo I inhibitor) or VP-16 (a Topo II inhibitor) in KFr cells, a CDDP-resistant subline of the KF epithelial ovarian carcinoma cell line, but not in the parent KF cells. We subsequently assayed Topo protein levels and enzymatic activities in two sets of CDDP-sensitive and -resistant cell lines: KF and KFr, and HeLa and HeLa/CDDP. The levels of Topo I protein in the CDDP-resistant cells did not differ from those of their parent cell lines and were unaffected by exposure to CDDP. Topo I enzymatic activity, however, was 2- to 4-fold higher in the CDDP-resistant cell lines than in their respective parent cell lines. In contrast, higher levels of Topo IIα protein were observed both before and after CDDP exposure in the CDDP-resistant cells than in their controls. However, no difference in Topo II catalytic activity was observed between the CDDP-resistant and -sensitive cells.

Key words: Cisplatin — Drug-resistance — Topoisomerase I — Topoisomerase II — Cancer cells

Cisplatin (*cis*-diamminedichloroplatinum(II); CDDP) is one of the most active anticancer agents now used. Despite its broad clinical applications,[1] however, intrinsic or acquired resistance to CDDP often culminates in chemotherapeutic failure.[2] Several mechanisms have been proposed for CDDP resistance including decreased accumulation, increased cellular detoxification by proteins such as glutathione, and increased DNA repair.[3,4] To overcome resistance to CDDP, the use of other drugs given in combination seems to be essential. Experimental studies on the combined effects of CDDP and other cytotoxic agents can provide information on the most appropriate sequence.

DNA topoisomerases are currently of interest as targets for cancer chemotherapy.[5] Etoposide, epipodophyllotoxin or VP-16, which inhibits the activity of topoisomerase II (Topo II), is used as a second-line treatment in patients whose cancers are resistant to CDDP.[6,7] VP-16 shows a synergistic enhancement of cisplatin cytotoxicity and has high activity against a cell line that is resistant to CDDP.[8] Camptothecin (CPT) inhibits Topo I through the formation of stable Topo I-DNA cleavable complexes.[9] CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, a semisynthetic water-soluble derivative of camptothecin, has potent antitumor activities.[10] Clinical trials of a combination of CDDP and CPT-11 are under way.[11] We previously reported a collateral sensitivity of cells to 7-ethyl-10-hydroxycamptothecin (SN-38), an active metabolite of CPT-11, and a synergistic interaction of CDDP and SN-38 against CDDP-resistant cells.[12] However, the biological basis of these phenomena in CDDP-resistant cells is not clear. Our present objective was to elucidate the combined effect of CDDP and Topo inhibitors on another CDDP-resistant cell line as well as to investigate the biological factors involved in the sensitivity to these anti-cancer agents.

MATERIALS AND METHODS

Cell lines and culture HeLa cells (ATCC CCL-2) and a CDDP-resistant subline, HeLa/CDDP cells were maintained in Eagle's minimum essential medium (Bio Whittaker, Walkersville, MD) containing 2 m*M* glutamine, 100 IU/ml of penicillin, and 10% fetal bovine serum (FBS) (Cansera, Ontario, Canada) at 37°C in a humidified incubator with 95% air and 5% CO_2. HeLa/CDDP cells were established by continuous exposure of HeLa cells to stepwise escalating concentrations of CDDP for 6 months.[12] HeLa/CDDP cells showed more than 8-fold resistance to CDDP compared to the parent cells (Table I). KF cells, epithelial ovarian carcinoma cell line, and a CDDP-resistant subline, KFr[13] (kindly provided by Dr. Kikuchi, National Defense Medical College) were maintained in RPMI 1640 medium (Bio Whittaker) under the same conditions.

[1] To whom correspondence should be addressed.

Table I. Cytotoxicity of Anticancer Agents toward CDDP-sensitive and -resistant Cells

Agent	IC_{50} (μM)		Resistance factor	IC_{50} (μM)		Resistance factor
	KF	KFr		HeLa	HeLa/CDDP	
CDDP	0.5	2.4	4.8	1.1	9.0	8.2
VP-16	2.8	2.4	0.9	1.9	2.0	1.1
SN-38	3.8×10^{-3}	4.4×10^{-3}	1.2	38×10^{-3}	8.0×10^{-3}	0.2

Resistance factor is the IC_{50} of KFr cells divided by the IC_{50} of KF cells, or the IC_{50} of HeLa/CDDP cells divided by the IC_{50} of HeLa cells.

Drug and chemicals The drugs were obtained from the following sources: CDDP and VP-16, Nippon Kayaku, Tokyo; SN-38, Yakult, Tokyo. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO.

Sensitivity of KF and KFr cells to anticancer agents The sensitivity of KF and KFr cells to anticancer agents was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.[14] Briefly, cells were diluted with culture medium to the seeding density, suspended in 96-well tissue culture plate (120 μl, containing 10^4 cells, per well) (Sumitomo Bakelite, Tokyo), and preincubated at 37°C for 4 h. Cells were incubated for 96 h with 30 μl of CDDP (0.1–10 μM), VP-16 (0.01–100 μM), or SN-38 (0.1–100 nM) to obtain a dose-response curve for each drug. For combination assays, cells were treated with 15 μl of various concentrations of CDDP and either VP-16 or SN-38. Each plate had one control column (8 wells) containing medium without cells and a second column containing cells without drugs. After incubation for 92 h, 20 μl of MTT (2.5 mg/ml) was added to each well and the plates were further incubated for 4 h. One hundred and fifty microliters of dimethylsulfoxide was added and the plates were shaken vigorously on a plate-shaker to solubilize the MTT-formazan product. Absorbance at 570 nm was measured with a microplate reader, Model 450 (Bio-Rad, Richmond, CA).

Dose-response curves were plotted on a semi-log scale as a percentage of the control cell number, which was obtained from the samples containing cells without drugs. The resistance factor of each anticancer agent was defined as IC_{50}-KFr/IC_{50}-KF. The effects of CDDP, in combination with either VP-16 or SN-38, on KF and KFr cells at the IC_{50} were analyzed using an improved isobologram method.[15]

Detection of Topo proteins Topo proteins were detected by Western blot analysis before (0 h) and after (24 h, 48 h, 72 h) continuous exposure of each cell line to CDDP at the IC_{50}. Cells were harvested by trypsinization, sonicated briefly, solubilized on ice in a lysis buffer [50 mM Tris-HCl, 125 mM NaCl, 0.1% NP40, 5 mM ethylenediaminetetraacetic acid (EDTA), 50 mM NaF, 0.1% phenylmethylsulfonyl fluoride (PMSF), and protease inhibitor], and centrifuged at 25,000g for 5 min. The total protein concentration in the supernatant was measured by Bradford's method.[16] Protein (60 μg) was boiled in SDS buffer for 5 min and loaded onto 4–20% SDS gradient gels.[17] Following SDS-polyacrylamide gel electrophoresis, the proteins were transferred to a nitrocellulose membrane at 30 volts for 90 min.

The membrane was incubated for 2 h with anti-Topo antibody. The antibodies used were: anti-Topo I, a sclerosis patient's serum (1 : 250, TopoGen, Inc., Columbus, OH) and anti-Topo II, a mouse monoclonal antibody against human Topo IIα (1 : 1000, TopoGen, Inc.). The membrane was rinsed with phosphate buffer solution (PBS) and incubated with peroxidase-conjugated sheep anti-human IgG, or goat anti-mouse IgG, respectively, as a second antibody. ECL detection (Amersham International, Buckinghamshire, UK) was used for visualization.

Extraction of nuclear enzymes The extraction of nuclear enzymes was conducted as described by Crespi *et al.*[18] In brief, log-phase cells were collected by trypsinization, pelleted by centrifugation at 150g for 10 min and washed 3 times with ice-cold PBS. Cell pellets were resuspended in extraction buffer [150 mM NaCl, 1 mM KH_2PO_4, 5 mM $MgCl_2$, 1 mM EDTA, 0.2 mM dithiothreitol (DDT), 1 mM PMSF, pH 6.4] (2×10^7/ml), mixed gently, and allowed to stand at 0°C for 20 min. Lysis was achieved by pipeting the suspension 50 times. Extraction of nuclear enzymes was performed by addition of an equal volume of 850 mM NaCl nucleus buffer to give a final NaCl concentration of 500 mM, and the samples were mixed using a rotator at 4°C for 120 min. The mixture was centrifuged at 16,000g for 20 min at 4°C and the protein concentration in the supernatant was measured.[16]

Topo I and Topo II catalytic activity Topo I activity was assayed by measuring the relaxation of supercoiled plasmid DNA.[19] The buffer used was 10 mM Tris-HCl (pH 7.9), 1 mM HCl, 150 mM NaCl, 0.1% bovine serum albumin (BSA), 0.1 mM spermidine, and 5% glycerol. Relaxation was carried out by incubating a 1 μl sample for 30 min at 37°C. Reactions were terminated by the

addition of 2 μl of 3% SDS, 0.3% bromophenol blue, and 30% glycerol. Samples were separated by electrophoresis on 1% agarose gel in 89 m*M* Tris-borate and 2 m*M* EDTA (pH 8.3) at 50 V for 120 min. Gels were stained with ethidium bromide (1.0 mg/ml) for 30 min and destained for 30 min in H_2O.

Topo II activity was assayed using the kinetoplast DNA decatenation assay.[20,21] The buffer used was 50 m*M* Tris-HCl (pH 8.0), 120 m*M* KCl, 10 m*M* $MgCl_2$, 0.5 m*M* ATP, 0.5 m*M* DTT, and 3% BSA. The procedures were essentially the same as for the Topo I assay, except that the gels and running buffer contained ethidium bromide, and electrophoresis was performed at 100 V for 60 to 90 min.

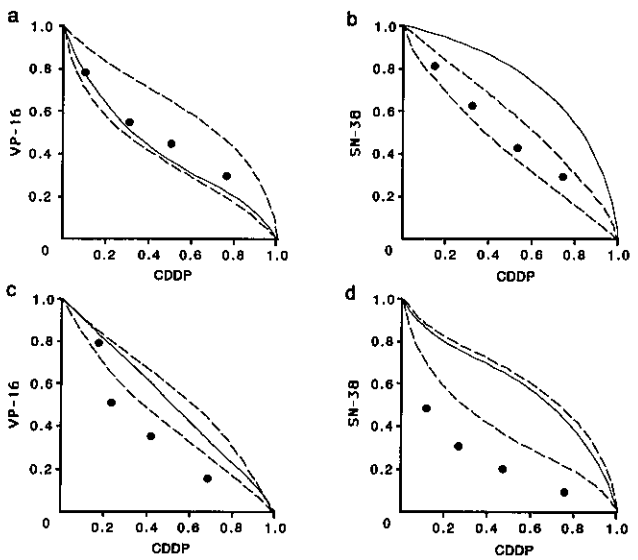

Fig. 1. Isobolograms of CDDP at IC_{50}, based on dose-response curves, in combination with VP-16 (a) or SN-38 (b) for KF cells, and of CDDP in combination with VP-16 (c) or SN-38 (d) for KFr cells. The abscissa shows the ratio of concentration to the IC_{50} for CDDP, and the ordinate represents that for VP-16 or SN-38. The solid line represents mode I, and the two broken lines represent mode II.

RESULTS

Sensitivity of KF and KFr cells to anticancer agents

Compared with KF cells, the KFr cells showed a 4.8-fold resistance to CDDP (Table I). In contrast, there was no significant difference between these two cell lines in their sensitivity to VP-16 and SN-38. Based on dose-response curves of these cell lines to CDDP in combination with VP-16 or SN-38, isobolograms at IC_{50} were plotted (Fig. 1). For KF cells, the combined data points fell within an envelope surrounded by three lines (Fig. 1, a and b), suggesting that the exposure of KF cells to CDDP combined with VP-16 or SN-38 produced only additive effects on these cells. In contrast, for KFr cells, the data points were below the envelopes (Fig. 1, c and d). This suggested that, for KFr cells, the combination of CDDP and SN-38 was supra-additive (synergistic), and the combination of CDDP and VP-16 was partially synergistic.

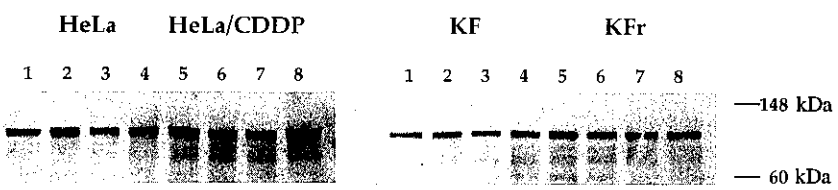

Fig. 2. Western blot analysis of Topo I. HeLa (left lanes, 1–4), HeLa/CDDP (left lanes, 5–8), KF (right lanes, 1–4), and KFr (right lanes, 5–8) cells were assayed before and after exposure to CDDP at the IC_{50}. Cells were exposed for: lanes 1 and 5, 0 h; lanes 2 and 6, 24 h; lanes 3 and 7, 48 h; lanes 4 and 8, 72 h.

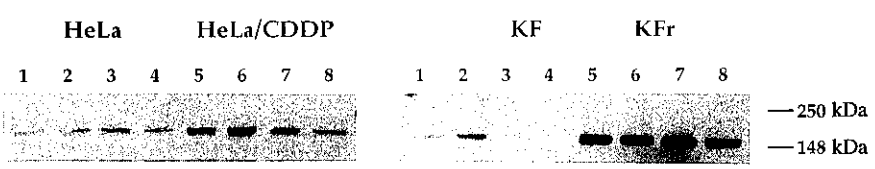

Fig. 3. Western blot analysis of Topo IIα. HeLa (left lanes, 1–4), HeLa/CDDP (left lanes, 5–8), KF (right lanes, 1–4), and KFr (right lanes, 5–8) cells were assayed before and after exposure to CDDP at the IC_{50}. Cells were exposed for: lanes 1 and 5, 0 h; lanes 2 and 6, 24 h; lanes 3 and 7, 48 h; lanes 4 and 8, 72 h.

Topo protein contents The baseline levels of Topo I protein in CDDP-resistant cells did not differ from those in their parent cells (Fig. 2). Furthermore, the amount of Topo I protein did not change significantly in any cell line after exposure to CDDP. In contrast, the levels of Topo IIα protein in CDDP-resistant cells were significantly greater than that in the parent cells prior to CDDP exposure (Fig. 3). The amount of Topo IIα protein after exposure to CDDP in the CDDP-resistant cells was consistently greater than that in the parent cells.

Topo I and Topo II catalytic activity in CDDP-resistant and -susceptible cell lines Topo I activity in each of the CDDP-resistant cell lines was greater than in their respective parent cell lines; the increase was 4-fold for HeLa/CDDP cells versus HeLa cells, and 2-fold for KFr cells versus KF cells (Fig. 4). In contrast, there was no difference in Topo II activity between the CDDP-resistant and the parent cells (Fig. 5).

DISCUSSION

The present study showed that CDDP combined with SN-38, a Topo I inhibitor, exhibited a synergistic effect against KFr cells, which were resistant to CDDP, although this agent exhibited only an additive effect against KF cells, the parent cells. In our previous study,[12] a synergism between CDDP and SN-38 was found in HeLa/CDDP cells, but not in HeLa cells. It was interesting to find that the synergistic enhancement of CDDP cytotoxicity by Topo I inhibitor was observed only in CDDP-resistant cells. As regards Topo II, a partial synergism between CDDP and VP-16, a Topo II inhibitor, was found in KFr cells, but not in KF cells. However, synergistic enhancement of CDDP cytotoxicity by VP-16 was not observed in either HeLa or HeLa/CDDP cells. It is therefore suggested that only a Topo I inhibitor enhances CDDP cytotoxicity in CDDP-resistant cells, al-

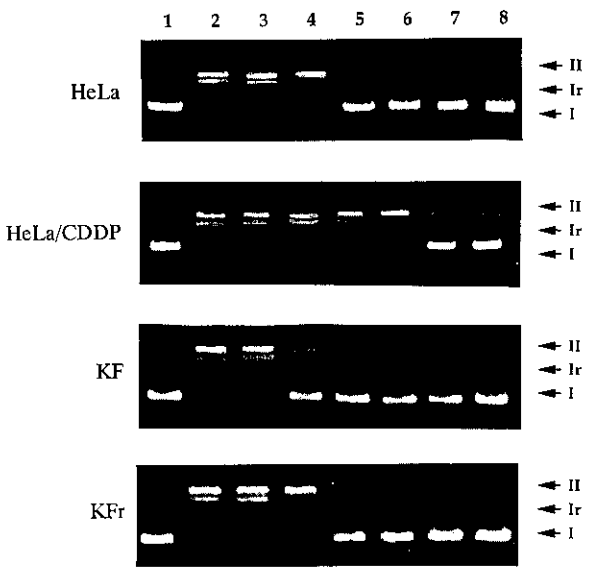

Fig. 4. Topo I catalytic activity in HeLa, HeLa/CDDP, KF, and KFr cells. For HeLa and HeLa/CDDP cells, the amounts of nuclear extracts assayed were: 0 ng (lane 1), 2, 800 ng (lane 2), 400 ng (lane 3), 200 ng (lane 4), 100 ng (lane 5), 50 ng (lane 6), 25 ng (lane 7), or 12.5 ng (lane 8). For KF and KFr cells, the amounts were: 0 ng (lane 1), 2, 200 ng (lane 2), 100 ng (lane 3), 50 ng (lane 4), 25 ng (lane 5), 12.5 ng (lane 6), 6.3 ng (lane 7), and 3.1 ng (lane 8).

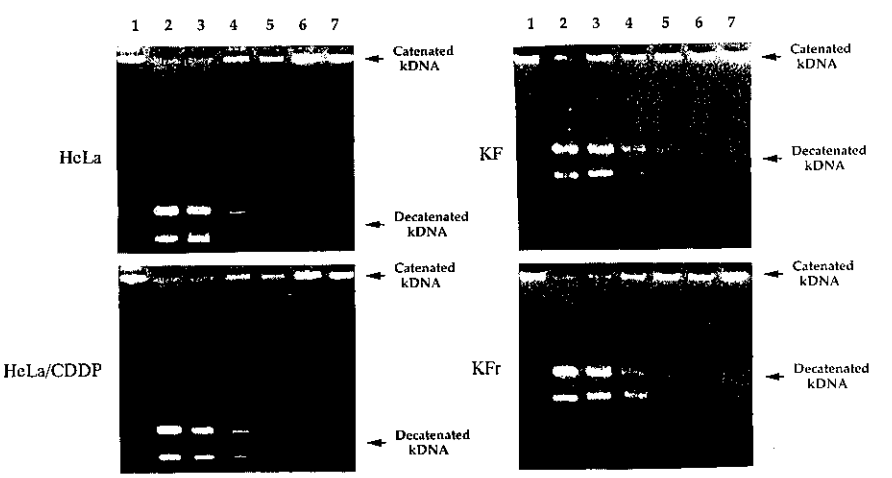

Fig. 5. Topo II catalytic activity in HeLa, HeLa/CDDP, KF, and KFr cells. The amounts of nuclear extracts added were the same as shown in Fig. 4, except for the lowest amounts (lane 8 of Fig. 4).

though the basis for this is still not clear. The present results, together with our previous finding, suggest a role for Topos in the CDDP-resistance of these cell lines and imply the existence of a quantitative and/or qualitative change of Topos in CDDP-resistant cells.

Topo I activity in both CDDP-resistant cell lines exceeded that in the respective parent cells, despite the similar levels of Topo I protein in each pair. The enhanced Topo I activity may contribute to a collateral sensitivity to a Topo I inhibitor, which was found in HeLa/CDDP, in agreement with a previous report.[22] However, KFr did not show such collateral sensitivity in spite of the synergism between CDDP and SN-38. Based on these phenomena, it is difficult to explain the mechanisms of synergy between CDDP and Topo I inhibitor in terms of Topo I activity. A potential mechanism to explain the synergism between CDDP and Topo I inhibitors has been proposed, involving an increase of DNA interstrand cross-linking by the simultaneous exposure to CDDP and Topo I inhibitor, and an enhancement of the activity of Topo I inhibitors by high doses of CDDP.[23] Although these findings do not explain why the enhancement of CDDP cytotoxicity by a Topo I inhibitor was observed only in the CDDP-resistant cells in our study, the enhanced Topo I activity could, at least in part, contribute to the enhancement of CDDP cytotoxicity in CDDP-resistant cells as compared with their parent cells.

Topo II is involved in many aspects of DNA metabolism, including transcription, replication, recombination, and chromosome segregation.[5] Among the Topo II isoforms, Topo IIα is considered to be more sensitive to drug-mediated inhibition than Topo IIβ.[24] To explain the synergism between CDDP and Topo II inhibitors, several mechanisms have been proposed, such as the enhancement of interstrand cross-links,[25] and enhanced replicative bypass of platinum-DNA adducts.[26] Interestingly, the protein levels of Topo IIα were higher in the CDDP-resistant cells than in their parent cells both before and after CDDP exposure, but there was no corresponding difference in Topo II catalytic activity in the present study. Other investigators have also found no relationship between Topo IIα protein levels and catalytic activity.[27,28] A partial synergism between CDDP and Topo II inhibitor in KFr cells, could, in part, be attributed to the above-mentioned biochemical events. But, the parent KF cells and both HeLa and HeLa/CDDP cells did not show the synergism. Therefore, our finding of increased levels of Topo IIα in CDDP-resistant cell lines could be regarded as a characteristic of acquired CDDP-resistant cells.

Although altered protein expressions and activities of Topos could not clearly explain the synergistic interaction of CDDP and Topo inhibitors, cisplatin-resistant cell lines tested in the present study have enhanced Topo I activity and increased Topo IIα protein, which may be consequences of qualitative changes of the enzymes. Alterations of Topo protein expression or activity may contribute to the resistant phenotype of these cells.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

(Received August 20, 1997/Accepted October 1, 1997)

REFERENCES

1) Loehrer, P. J. and Einhorn, L. H. Drugs five years later. Cisplatin. *Ann. Intern. Med.*, **100**, 704–713 (1984).
2) Ozols, R. F. and Young, R. C. Chemotherapy of ovarian cancer. *Sem. Oncol.*, **11**, 251–263 (1984).
3) Scanlon, K. J., Kashani-Sabet, M., Miyachi, H., Sowers, L. C. and Rossi, J. Molecular basis of cisplatin resistance in human carcinomas; model systems and patients. *Anticancer Res.*, **9**, 1301–1312 (1989).
4) Andrews, P. A. and Howell, S. B. Cellular pharmacology of cisplatin: perspectives on mechanisms of acquired resistance. *Cancer Cells*, **2**, 35–43 (1990).
5) Drlica, K. and Franco, R. J. Inhibitors of DNA topoisomerases. *Biochemistry*, **27**, 2253–2259 (1988).
6) Cain, J. M., Collins, C., Petersdorf, S., Figge, D. C., Tamimi, H. K., Greer, B. E. and Livingston, R. B. Phase II study of high-dose cisplatin, etoposide, and cyclophosphamide for refractory ovarian cancer. *Am. J. Obstet. Gynecol.*, **174**, 1688–1694 (1996).
7) Zanaboni, F., Scarfone, G., Presti, M., Maggi, R., Borello, C. and Bolis, G. Salvage chemotherapy for ovarian cancer recurrence: weekly cisplatin in combination with epirubicin or etoposide. *Gynecol. Oncol.*, **43**, 24–28 (1991).
8) Fujiwara, Y., Sugimoto, Y., Kasahara, K., Bungo, M., Yamakido, M., Tew, K. D. and Saijo, N. Determinants of drug response in a cisplatin-resistant human lung cancer cell line. *Jpn. J. Cancer Res.*, **81**, 527–535 (1990).
9) Hertzberg, R. P., Caranfa, M. J. and Hecht, S. M. On the mechanism of topoisomerase I inhibition by camptothecin: evidence for binding to an enzyme-DNA complex. *Biochemistry*, **28**, 4629–4638 (1989).
10) Kunimoto, T., Nitta, K., Tanaka, T., Uehara, N., Baba, H., Takeuchi, M., Yokokura, T., Sawada, S., Miyasaka, T. and Mutai, M. Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. *Cancer Res.*, **47**, 5944–5947 (1987).

11) Masuda, N., Fukuoka, M., Takada, M., Kusunoki, Y., Negoro, S., Matsui, K., Kudoh, S., Takifuji, N., Nakagawa, K. and Kishimoto, S. CPT-11 in combination with cisplatin for advanced non-small-cell lung cancer. *J. Clin. Oncol.*, **10,** 1775–1780 (1992).
12) Minagawa, Y., Kigawa, J., Ishihara, H., Itamochi, H. and Terakawa, N. Synergistic enhancement of cisplatin cytotoxicity by SN-38, an active metabolite of CPT-11, for cisplatin-resistant HeLa cells. *Jpn. J. Cancer Res.*, **85,** 966–971 (1994).
13) Kikuchi, Y., Miyauchi, M., Kizawa, I., Oomori, K. and Kato, K. Establishment of cisplatin-resistant human ovarian cancer cell line. *J. Natl. Cancer Inst.*, **77,** 1181–1185 (1986).
14) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods,* **65,** 55–63 (1983).
15) Kano, Y., Suzuki, K., Akutsu, M., Suda, K., Inoue, Y., Yoshida, M., Sakamoto, S. and Miura, Y. Effects of CPT-11 in combination with other anti-cancer agents in culture. *Int. J. Cancer,* **50,** 604–610 (1992).
16) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72,** 248–254 (1976).
17) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* **227,** 680–685 (1970).
18) Crespi, M. D., Ivanier, S. E., Genovese, J. and Baldi, A. Mitoxantrone affects topoisomerase activities in human breast cancer cells. *Biochem. Biophys. Res. Commun.*, **136,** 521–528 (1986).
19) Liu, L. F. and Miller, K. G. Eukaryotic DNA topoisomerases: two forms of type I DNA topoisomerases from HeLa nuclei. *Proc. Natl. Acad. Sci. USA,* **78,** 3487–3491 (1981).
20) Marini, J. C., Miller, K. G. and Englund, P. T. Decatenation of kinetoplast DNA by topoisomerases. *J. Biol. Chem.*, **255,** 4976–4979 (1980).
21) 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).
22) Kotoh, S., Naito, S., Yokomizo, A., Kumazawa, J., Asakuno, K., Kohno, K. and Kuwano, M. Increased expression of DNA topoisomerase I gene and collateral sensitivity to camptothecin in human cisplatin-resistant bladder cancer cells. *Cancer Res.*, **54,** 3248–3252 (1994).
23) Fukuda, M., Nishio, K., Kanazawa,F., Ogasawara, H., Ishida, T., Arioka, H., Bojanowski, K., Oka, M. and Saijo, N. Synergism between cisplatin and topoisomerase I inhibitors, NB-506 and SN-38, in human small cell lung cancer cells. *Cancer Res.*, **56,** 789–793 (1996).
24) van der Zee, A. G. J., de Jong, S., Keith, W. N., Hollema, H., Boonstra, H. and de Vries, E. G. E. Quantitative and qualitative aspects of topoisomerase I and IIα and β in untreated and platinum/cyclophosphamide treated malignant ovarian tumors. *Cancer Res.*, **54,** 749–755 (1994).
25) de Jong, S., Timmer-Bosscha, H., de Vries, E. G. E. and Mulder, N. H. Effect of novobiocin on cisplatin cytotoxicity and DNA interstrand cross-link formation in a cisplatin-resistant, small-cell lung carcinoma cell line. *Int. J. Cancer,* **53,** 110–117 (1993).
26) Mamenta, E. L., Poma, E. E., Kaufmann, W. K., Delmastro, D. A., Grady, H. L. and Chaney, S. G. Enhanced replicative bypass of platinum-DNA adducts in cisplatin-resistant human ovarian carcinoma cell lines. *Cancer Res.*, **54,** 3500–3505 (1994).
27) Barret, J. M., Calsou, P., Larsen, A. K. and Salles, B. A cisplatin-resistant murine leukemia cell line exhibits increased topoisomerase II activity. *Mol. Pharmacol.*, **46,** 431–436 (1994).
28) Brown, G. A., McPherson, J. P., Gu, L., Hedley, D. W., Toso, R., Deuchars, K. L., Freedman, M. H. and Goldenberg, G. J. Relationship of DNA topoisomerase IIα and β expression to cytotoxicity of antineoplastic agents in human acute lymphoblastic leukemia cell lines. *Cancer Res.*, **55,** 78–82 (1995).