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A Cisplatin-Resistant Murine Leukemia Cell Line Exhibits Increased Topoisomerase II Activity

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SUMMARY

cis-Dichlorodiammineplatinum(II) (CDDP) resistance in L1210/10 murine leukemia cells is multifactorial and involves decreased drug uptake, increased glutathione content, and enhanced DNA repair activity. We show here that 0.35 M NaCl nuclear extracts from L1210/10 cells possess an approximately 3-fold increase in DNA topoisomerase II activity, compared with parental L1210 cells, as measured by decatenation of kinetoplast DNA. No difference in topoisomerase I activity is observed between the two cell lines. Immunoblot analysis of topoisomerase II protein in resistant and sensitive cells suggests that the observed differences in topoisomerase II activity cannot be explained by differences in the level of protein expressed. L1210/10 cells are 2.5-fold more sensitive than L1210 cells to the cytotoxic effects of the topoisomerase II inhibitor 4′-(9-acridylamino)methanesulfon-*m*-anisidide. Sequential treatment with 4′-(9-acridylamino)methanesulfon-*m*-anisidide and CDDP leads to an additive cytotoxic effect of the two drugs in sensitive L1210 cells, as determined by colony formation in semi-solid medium. In contrast, the same treatment leads to a supra-additive effect in L1210/10 cells, which strongly suggests a role for topoisomerase II in the CDDP resistance of this cell line.

CDDP is a widely used drug in cancer chemotherapy. It exerts its toxicity by covalently binding to cellular nucleophiles, among which DNA represents the ultimate target (1). To circumvent CDDP toxicity, various mechanisms of cellular resistance are involved, including (i) impaired drug uptake, (ii) increased concentrations of secondary nonessential targets such as glutathione and metallothioneins, (iii) enhanced DNA repair, and (iv) the ability to tolerate unrepaired lesions in DNA (2–4). Cisplatin/DNA adducts are repaired either by recombination or by excision of the lesion. In terms of cell survival, the nucleotide excision-repair pathway appears to play a major role, because cells that are deficient in that pathway are highly sensitive to CDDP treatment (4).

Resistance to CDDP has been studied in numerous cell lines, including murine L1210 leukemia cells, from which the L1210/10 variant has been selected by continuous *in vitro* exposure to increasing concentrations of CDDP (5). The L1210/10 cell line exhibits multiple mechanisms of drug resistance (5). To show the involvement of DNA repair in L1210/10 cell resistance, Eastman and co-workers (6) have analyzed the cell repair activity with a damaged reporter gene cloned into a plasmid. L1210/10 cells transfected with CDDP-treated pRSVcat plasmid showed a higher level of expression of chloramphenicol acetyltransferase, compared with sensitive L1210 cells (6). However, the overall *cat* gene expression of untreated plasmid in L1210/10 cells was about 10-fold lower than in L1210 transfectants (6). In contrast, when DNA repair was analyzed by an *in vitro* assay that allowed quantification of repair synthesis in a CDDP-damaged plasmid, we found no difference in repair capacity between L1210/10 and L1210 protein extracts (7). During the course of these experiments, we noticed a difference in plasmid catenation when plasmid DNA was incubated in the presence of protein extracts from resistant and sensitive cells. Based upon these results, we hypothesized that the topoisomerase activity might be different in the two cell lines.

Two major topoisomerases, topo I and topo II, have been identified in eukaryotic cells (8). These nuclear enzymes function by forming protein-linked DNA strand breaks that act as transient gates for the passage of another DNA strand. Topo I breaks one strand of duplex DNA and allows the unbroken strand to pass through the enzyme-linked strand break, resulting in DNA relaxation. Topo II breaks both strands of duplex DNA and can thus relax supercoiled DNA and catenate or decatenate DNA circles. Moreover, topo II exists in two structurally similar forms, of 170,000 kDa (topo IIα) and 180,000 kDa (topo IIβ) (9). The biological consequences of topoisomerase activity consist of relieving DNA from topological constraints due to transcription, replication, and chromosomal

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ABBREVIATIONS: CDDP, *cis*-dichlorodiammineplatinum(II); *m*-AMSA, 4′-(9-acridylamino)methanesulfon-*m*-anisidide; kDNA, kinetoplast DNA; CI, combination index; topo I, DNA topoisomerase type I; topo II, DNA topoisomerase type II; PCV, packed cell volume(s); SDS, sodium dodecyl sulfate.

disjunction or segregation. Futhermore, these enzymes represent the principal targets for a number of important antitumor agents (8, 10).

This study details the comparison of nuclear topo II activity from resistant L1210/10 and parental L1210 cells. The results show that the topo II-dependent decatenation of kDNA by nuclear extracts from L1210/10 cells showed an approximately 3-fold increase, compared with extracts from the sensitive parental cells. This modulation of topo II activity is discussed with reference to the known mechanisms of cisplatin resistance in L1210 leukemia cells.

Experimental Procedures

Materials. CDDP (a gift from Roger Bellon Cie), *m*-AMSA (generously provided by Parke Davis), and ethidium bromide (Sigma) were prepared as stock solutions. Anti-topo II antiserum was recovered from rabbits after four subcutaneous injections at 30-day intervals with 300 μg of purified topo II from *Saccharomyces cerevisiae*. Highly catenated kDNA was purified from *Trypanosoma cruzi* (kindly provided by Dr. G. Riou, Institut Gustave Roussy, Villejuif, France) after DNA extraction and sucrose sedimentation, as described (11).

Cell lines and culture. L1210 parental and L1210/10 CDDP-resistant cells (a gift from Dr. Eastman, Dartmouth Medical School, Hanover, NH) (5) were routinely maintained in RPMI 1640 medium (GIBCO-BRL) supplemented with 10% fetal calf serum, 2×10^5 units/liter penicillin, and 50 mg/liter streptomycin. Subculture every 2–3 days maintained the cells in the exponential growth phase. The stability of the resistant phenotype was confirmed by growing the L1210/10 cells in the presence of 5 μg/ml CDDP (a nontoxic concentration, as judged from the growth curve). Cells were regularly tested and found to be free of contamination with *Mycoplasma* (*Mycoplasma* detector kit; Boehringer).

Nuclear extracts. Nuclear extracts were obtained according to the method of Miller *et al.* (12), with minor modifications. Leukemia cells (about 6×10^5 cells/ml) were collected by centrifugation (10 min at 800 × *g*) and the pellets were washed twice with 3 PCV of phosphate-buffered saline and then with 1 PCV of phosphate-buffered saline containing 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, and 1 μg/ml soybean trypsin inhibitor. Cells were resuspended in 1 PCV of hypotonic buffer (5 mM KH_2PO_4, pH 7, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 10 mM 2-mercaptoethanol, 10 μg/ml soybean trypsin inhibitor, 50 μg/ml leupeptin) and incubated for 20 min. The swollen cells were disrupted by 20 strokes of a Dounce B homogenizer and the nuclei were pelleted by centrifugation at 800 × *g* for 10 min. The pelleted nuclei were resuspended in 1 PCV of nuclei extraction buffer (hypotonic buffer with 0.35 M NaCl, 10 μg/ml aprotinin, and 10% glycerol) and incubated for 30 min, with gentle stirring. After centrifugation at 800 × *g*, the nuclear extract (i.e., the supernatant) was removed, immediately frozen with 30% glycerol (final concentration), and stored at −80°. Protein concentrations of the nuclear extracts were measured by the microassay procedure, using Bio-Rad protein assay dye (Bio-Rad Laboratories). All extracts from sensitive and resistant cells were adjusted to the same protein concentration by dilution with nuclei extraction buffer.

Decatenation assay. A 15-μl reaction mixture contained 50 mM Tris·HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 165 mM KCl, 1 mM ATP, and 200 ng of kDNA, as described (13). The reaction was initiated at 30° by the addition of nuclear extract and was arrested by the addition of 1% SDS, 0.5% bromophenol blue, 30% glycerol. The concentration of undiluted nuclear extracts in the reaction mixture was adjusted to 1.7 mg/ml. The samples were electrophoresed in 1% agarose gels at 6 V/cm for 4 hr, in TBE buffer (89 mM Tris-borate, pH 8.0, 2 mM EDTA). Photographic negatives of the ethidium bromide-stained agarose gels were scanned with a scanning laser densitometer (Biocom, Les Ulis, France), and the quantity of liberated minicircles was determined as a percentage of total kDNA. Results are expressed in arbitrary units, and the ratio of activity in L1210/10 cells to that in L1210 cells was used for comparison.

Comparative amounts of immunoreactive topo II in nuclear extracts. Samples were not boiled before loading onto SDS-polyacrylamide gels, because this has been reported to result in loss of the 180-kDa isoform of topo II (13). Nuclear extracts, with equalized protein contents, were electrophoresed in 7.5% SDS-polyacrylamide gels at 5 mA overnight and were transferred to nitrocellulose filters (Hybond C Extra; Amersham), in 50 mM Tris, 380 mM glycine, 0.1% SDS, 20% methanol, by electroblotting with a Trans-Blot cell (Bio-Rad) at 110 mA for 5 hr. The blots were blocked with 10% dried milk in TBS-T (10 mM Tris·HCl, pH 7.4, 0.9% NaCl, 0.3% Tween-20). The membranes were further incubated for 3 hr at 22° with an antibody directed against yeast topo II (1/500), rinsed three times (10 min for each wash) with TBS-T with 1% dried skim milk, treated with horseradish peroxidase-conjugated secondary antibody, and developed according to the manufacturer's specification (enhanced chemiluminescence kit; Amersham). Autoradiograms were scanned with a Biocom densitometer, and topo II protein levels were quantified. Results are expressed in arbitrary units, and the L1210/10 to L1210 ratio was used for comparison. A linear relationship existed between the value of the integration units and the amount of protein extract loaded on the gel in the range of 75–300 μg of protein (data not shown). We therefore used 150 μg of protein extracts for each cell line in subsequent experiments.

Topo I assay. Topo I activity was measured by the relaxation of supercoiled DNA. Incubation conditions with nuclear extract were as described above, except that 200 ng of supercoiled pBluscript KS^+ (Stratagene) plasmid was used as substrate, in the absence of ATP. The experiment was then performed as reported for the decatenation assay. Photographic negatives of the ethidium bromide-stained agarose gels were scanned, and the peak area of supercoiled DNA was determined.

Colony-forming assay. Cell survival was determined by colony formation in soft agar. Exponentially growing cells were exposed to drug for 3 hr (*m*-AMSA) or 24 hr (CDDP) at 37°. The drug was removed and the cells were washed once and then resuspended in 10 ml of culture medium containing 60 μM 2-mercaptoethanol. Inclusion of 60 μM 2-mercaptoethanol increases the efficiency of L1210 colony formation, as observed for other lymphoid cells. Cells were counted with a Coulter counter and diluted to obtain 300 cells/dish. This cell density ensured maximum sensitivity for a proportional response. Cell suspension (2.5 ml) was added to 0.4 ml of 2.2% Noble agar (Difco), mixed at 42°, and transferred to 35-mm dishes. The dishes were incubated at 37° in humidified 5% CO_2. After 13 days, cells were stained with tetrazolium salts and colonies greater than 125 μm were counted with an Accucount counter (Artek Co). Each treatment was assayed in triplicate and the individual values did not vary from the mean by >15%. Cloning efficiency was >90%.

Median effect analysis. This analysis was used to determined the nature of the interaction between CDDP and *m*-AMSA (14). With computer-assisted median effect analysis, we have calculated the CI as a function of the percentage cell kill by the drugs. CI values of less than or greater than 1 indicate synergy or antagonism, respectively, whereas a CI value of 1 indicates additivity of the drug effects. The CI was determined from colony-forming assays at increasing levels of cell kill. CDDP and *m*-AMSA were combined at a ratio equal to the ratio of the IC_{50} values for each drug determined by clonogenic assay. Five different dose combinations were used, and all experimental points were determined twice. L1210 cells were treated for 3 hr with *m*-AMSA and then exposed to CDDP for 24 hr. The platinum drug was then removed and the cells were plated as described for colony-forming assays. The cytotoxic effect of the combination was compared with the cytotoxicity of CDDP and *m*-AMSA alone, using a Biosoft (Cambridge, UK) computer system.

Results

Comparison of topo II activity in L1210 and L1210/10 cells. The strand-passing activity of topo II was measured by decatenation of kDNA. This reaction, resulting in the release

of double-stranded minicircles from the catenated kDNA network, is ATP dependent and specific for topo II. Fig. 1A shows the results obtained with various dilutions of 0.35 M NaCl nuclear extracts. An enhanced decatenation activity was observed with L1210/10 nuclear extracts. Quantification of free minicircles showed a 1.6-, 3.3-, 2.7-, and 1.8-fold increase in catalytic activity of L1210/10 cells, compared with sensitive L1210 cells, with extract dilutions of 1/10, 1/50, 1/75, and 1100, respectively. In a set of three independent experiments with five different nuclear extracts of L1210/10 cells diluted 120, we found a 2.8 ± 0.3-fold increase in catalytic activity. A similar 3-fold increase in the activity of nuclear extracts from L1210/10 cells was observed when the catalytic activity was determined in kinetic experiments (Fig. 1B).

Topo II protein levels in L1210 and L1210/10 cells. The increased topo II catalytic activity could result from an increased protein level in L1210/10 cells. Therefore, the levels of topo IIα and IIβ proteins in four independently prepared nuclear extracts were determined by Western blot analysis using polyclonal anti-topo II serum (Fig. 2). Densitometric analysis of the immunoblots showed no significant difference in the levels of topo II protein expressed in the two cell types. The ratio of sensitive to resistant cells was calculated to be 1.05 ± 0.07. Moreover, no variation in the ratio of topo IIα to topo IIβ was found (1.07 ± 0.15 and 1.20 ± 0.18 in resistant and sensitive cells, respectively). Therefore, the enhanced topo II activity was not due to an increase in topo II levels in L1210/10 cells.

Comparison of topo I activity in L1210 and L1210/10 cells. It was possible that the increase in topo II activity might be balanced by decreased topo I activity. We therefore determined topo I activity in both L1210/10 and L1210 nuclear extracts by quantitation of the relaxation of supercoiled plasmid DNA in the absence of ATP. The extracts contained more topo I than topo II activity, because higher dilutions of the extracts were required to determine their relative activities (Fig. 3A). Quantification of relaxed forms gave a ratio of 1.1 ± 0.2 for L1210/10 versus L1210 nuclear extracts for the dilutions tested, showing similar topo I activities in the two cell lines. Moreover, when the activity was determined in kinetic experiments, the topo I activity was the same with L1210/10 and L1210 nuclear extracts (Fig. 3B). Similar results were obtained with four independent extracts. Therefore, only the topo II activity appears to be affected in CDDP-resistant leukemia cells.

Drug sensitivity of L1210/10 and L1210 cells. Firstly, the resistance of L1210/10 cells to CDDP was verified by colony-forming assays after 24 hr of drug exposure (Fig. 4A). Comparison of the toxic doses allowing 50% survival (IC_{50}) of L1210/10 versus L1210 cells showed a 15-fold resistance factor, as reported previously (15). Secondly, because topo II was enhanced in resistant cells, its response to *m*-AMSA, a potent topo II inhibitor, was also tested (16). L1210/10 cells were 2.5-fold more sensitive to *m*-AMSA than were L1210 cells, as determined by clonogenic experiments after 3-hr drug treatments (Fig. 4B). This is also in agreement with preliminary results already reported (17).

Median effect analysis of the interaction between *m*-AMSA and CDDP. The increased topo II activity in L1210/10 cells could be partly responsible for CDDP resistance. This possibility was tested by the exposure of both sensitive and resistant cells to *m*-AMSA for 3 hr and then to CDDP for 24 hr. This treatment allowed us to study the effect of topo II inhibition on the sensitivity to CDDP in the two cell lines. Cell survival was determined by clonogenic assay. IC_{50} values for CDDP and *m*-AMSA were 0.65 ± 0.05 μM and 5.2 ± 0.3 nM, respectively, for the L1210 cells and 9.8 ± 0.4 μM and 2.1 ± 0.3 nM, respectively, for L1210/10 cells (Fig. 4). The median effect analysis used *m*-AMSA to CDDP molar ratios of 1:120 for the L1210 cell line and 1:5000 for the L1210/10 cell line, which

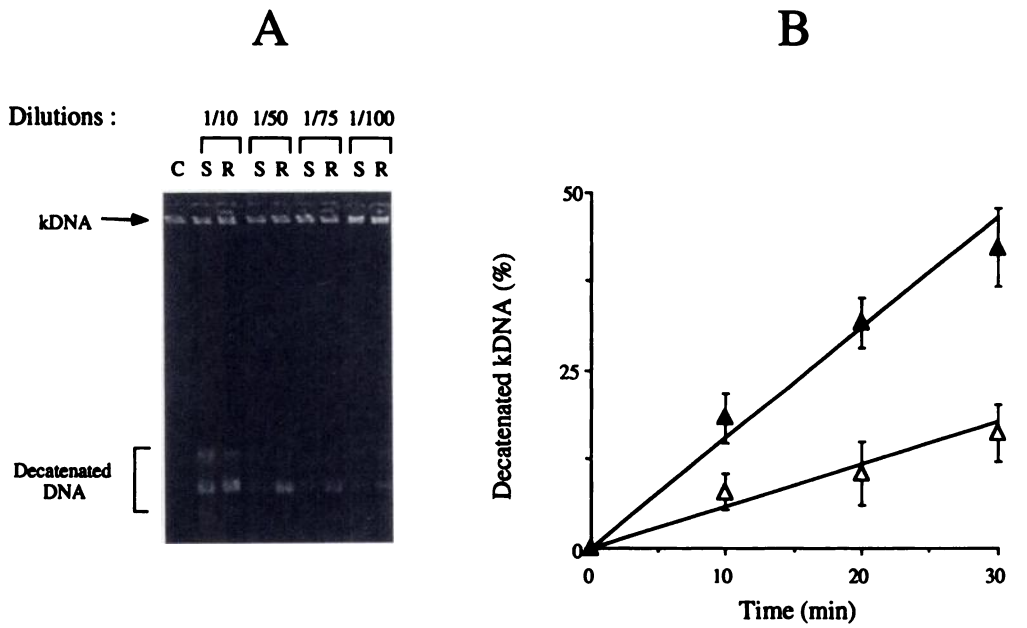

Fig. 1. Decatenation activity of topo II. Topo II activity in nuclear extracts from L1210 and L1210/10 cell lines was determined by decatenation of kDNA. A, Serial dilutions of nuclear extracts from L1210 (*S*) and L1210/10 (*R*) cell lines. (Lane C: kDNA incubated with nuclear extract buffer alone.) B, Topo II activity determined after different incubation times with nuclear extracts from L1210 (△) and L1210/10 (▲) cell lines. The dilution of nuclear extract was 1/20. The protein concentration of the extracts corresponded to 1.27 μg/assay. The quantity of free minicircles was determined as decribed in Experimental Procedures. Values are the mean of three independent experiments; *bars*, standard errors.

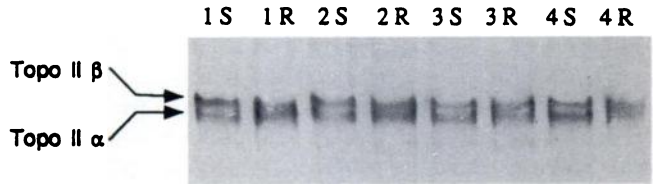

Fig. 2. Topo II protein levels. A Western blot of nuclear proteins from L1210 (*S*) and L1210/10 (*R*) cell lines using anti-topo II antiserum, as described in Experimental Procedures, is shown. Four independent extractions (*1–4*) are shown.

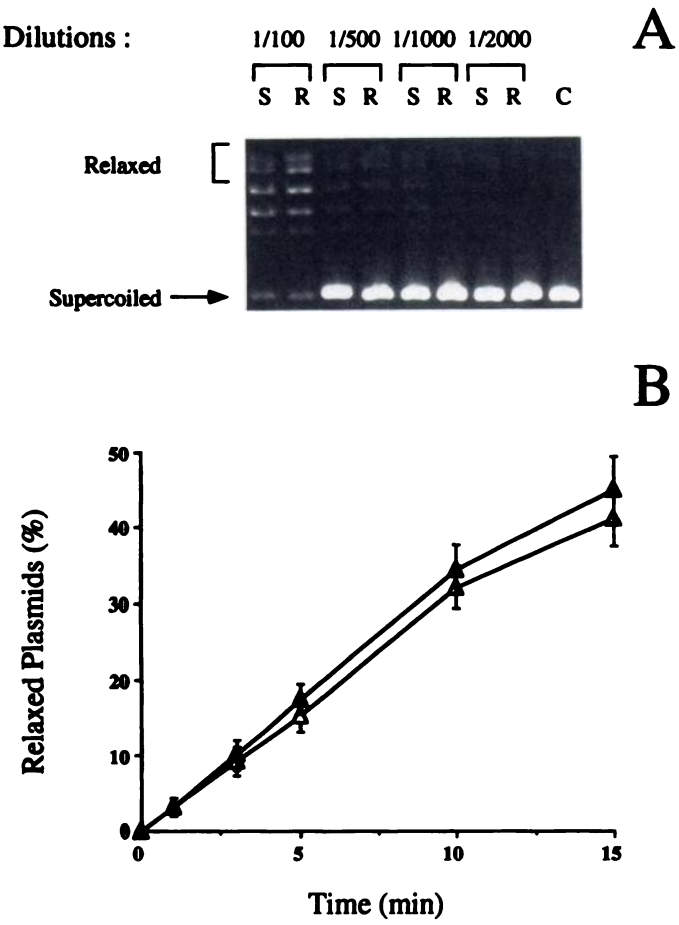

Fig. 3. Topo I activity. Topo I activity in nuclear extracts from L1210 and L1210/10 cell lines was determined by relaxation of supercoiled plasmid DNA. A, Serial dilutions of nuclear extracts from L1210 (*S*) and L1210/10 (*R*) cell lines were incubated for 5 min. (Lane C: supercoiled plasmid DNA incubated with nuclear extract buffer alone.) B, Topo I activity was tested four times at variable incubation times with nuclear extracts from L1210 (△) and L1210/10 (▲) cell lines. The dilution of nuclear extracts was 1/400. The protein concentration of the extracts corresponded to 64 ng/assay.

were approximately the ratios of the IC_{50} values for the drugs. Fig. 5 shows a plot of the CI as a function of the percentage cell kill for the interaction between *m*-AMSA and CDDP. Median effect analysis showed CI values of approximately 1 for the L1210 cell line, indicating additivity of *m*-AMSA and CDDP toxicity in these cells. In contrast, CI values for L1210/10 cells were about 0.5 in the range of 50% cell kill, suggesting a highly synergistic effect.

Discussion

Cellular resistance to antitumor agents represents a severe limitation to the chemotherapeutic treatment of cancer patients

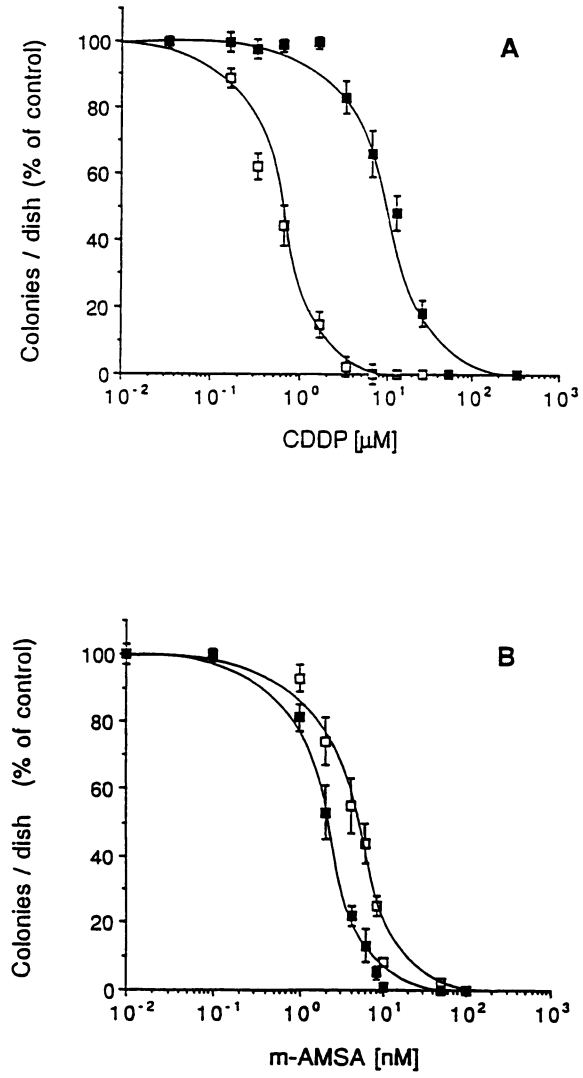

Fig. 4. Cellular toxicity of CDDP and *m*-AMSA, determined by cloning efficiency. Dose-survival curves for L1210 (□) and L1210/10 (■) cell lines after treatment with CDDP (A) or *m*-AMSA (B) are shown.

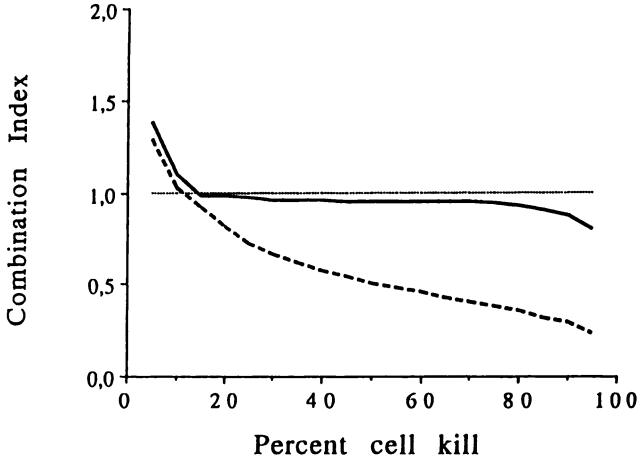

Fig. 5. Median effect analysis. CI plots for *m*-AMSA plus CDDP in L1210 (——) and L1210/10 (– – –) cell lines are shown.

with cytotoxic drugs. In an attempt to understand the molecular mechanisms underlying CDDP (cisplatin) resistance, various cell lines of different origins, including the L1210 murine leukemia cell line, have been used (17). A resistant variant,

L1210/10, was selected by growing the parental cells in the presence of increasing concentrations of CDDP. The resistant phenotype is multifactorial and exhibits decreased uptake, increased glutathione content, and increased DNA repair activity (5, 6). In addition to these modifications, our results show an increased topo II activity. The enhanced activity was not due to an increase in protein content, because immunoblot analysis showed that the levels of neither topo IIα nor topo IIβ were changed in nuclear extracts isolated from resistant L1210/10 cells. This result is consistent with earlier studies in which the determination of topo II mRNA by Northern blot analysis showed no difference between L1210 and L1210/10 cells (18).

The decatenation assay was performed at a high salt concentration (165 mM KCl), which is optimal for topo IIβ (19). In addition, when the topo II activity was determined at a lower salt concentration, which favors topo IIα activity, the difference between resistant and sensitive cell extracts was less pronounced. These results suggest that topo IIβ was altered in L1210/10 nuclear extracts. This form of topo II has been shown to be involved in the resistance to topo II inhibitors (20) and might also be associated with certain topological domains of chromatin (21). However, our assay system is not suitable to determine whether a difference in topo II content exists between the two cell lines at the level of the nuclear matrix. A closer examination of the Western blots indicated that the electrophoretic migration was slightly different with resistant cell extracts, compared with extracts from sensitive cells. This might suggest a difference in post-translational modification of topo II, which is reported to exist as a phosphoprotein (10). In addition to alterations of the level or sites of phosphorylation, differential poly(ADP)-ribosylation might also result in modifications of the catalytic activity (22).

The cellular effects of intercalative drugs such as *m*-AMSA and the epipodophyllotoxin derivatives etoposide and teniposide have been the subject of numerous studies. Cell lines resistant to these agents have a topo II that is affected either quantitatively (23–25), qualitatively (26–28), or both (21, 29). Moreover, decreased topo II activity might result from mutations in the topo II gene (26, 27). Whatever the modification(s) of topo II, the mechanism of resistance appears to be the result of a decrease in the formation of protein-linked DNA breaks (cleavable complexes), which are thought to be responsible for the cytotoxic effects of *m*-AMSA, etoposide, and teniposide (24, 30–32). Therefore, the collateral sensitivity of L1210/10 cells to *m*-AMSA is consistent with the higher topo II activity found in extracts from these cells.

Cells resistant to topo II inhibitors can, in turn, present collateral sensitivity (hypersensitivity) to CDDP (33, 34). A possible correlation between topo II activity and cellular resistance to alkylating agents was also found in cells that were resistant to nitrogen mustards (35) and in L1210/10 cells that were resistant to CDDP and exhibited elevated topo II activity (our results). The effect of topo II on CDDP or alkylating agent toxicity has already been explored by the use of combinations that include topo II inhibitors. However, contradictory results have been obtained with such combinations, depending upon both the inhibitor and the cellular model used (36–38). In addition, the mode of calculation of synergistic effects could be discussed (14). To determine the consequence of an interaction between *m*-AMSA and CDDP, we have calculated a CI. This calculation is based upon equations derived for interactions between two or more reversible enzyme inhibitors that obey Michaelis-Menten or Hill kinetics and has been extended to the use of antineoplastic agents. If the index is 1, the drug combination is thought to be additive; supra-additivity is indicated by an index of <1 and antagonism by an index of >1. In the case of L1210 cells, topo II inhibition did not increase CDDP toxicity (CI = 1). In contrast, the decreased resistance factor seen when L1210/10 cells were pretreated with *m*-AMSA (CI $<$ 1) corresponds to a synergistic effect. This result clearly indicates that the increased topo II activity in the L1210/10 cell line is somehow involved in the resistance of this line to CDDP.

Because topo II activity appears to be involved in CDDP resistance, what could the mechanism be? Topo II is known to play a role in replication, transcription, and recombination but an involvement in DNA repair is not clear. Because DNA repair appears to be involved in CDDP resistance (2, 17), the increased topo II activity could lead to a higher level of repair, particularly by the process of nucleotide excision. A simple effect of topo II activity on excision repair capacity can be ruled out, because no cross-resistance to UV light or Melphalan was found in the L1210/10 cell line (17). Conflicting results have been reported concerning the putative role of topo II in DNA repair, with some describing no effect (36, 39, 40) and others finding an effect (41, 42). In this respect, a discrepancy also exists with the results of Sheibani *et al.* (6), who reported an increased repair activity of CDDP-damaged plasmids after transfection into L1210/10 cells. This is in contrast to our previous results, which showed no difference using an *in vitro* repair assay with cell-free extracts (7). The two assays used are different in several aspects; (i) the kinetics of adduct removal are faster *in vitro* than *in vivo* (17, 43), (ii) the major lesion, which is the 1,2-(dG-G) intrastrand adduct, is repaired *in vivo* but not *in vitro* (17, 44, 45), and (iii) some proteins needed for the recognition of cisplatin/DNA damage could be lost during the preparation of cell-free extracts. On the other hand, if high levels of topo II are indeed involved in the resistance mechanism, then this cannot be tested with the *in vitro* repair assay, because high topo I activity leads to relaxation of the plasmids within a few minutes (Fig. 3 and Ref. 46).

Another possible mechanism of topo II in CDDP resistance may involve modification of adduct distribution. For instance, it has been reported that interstrand cross-links are involved in CDDP resistance (47). On the other hand, it has been shown that the superhelical density modulates the rate of conversion of poorly toxic, monofunctional adducts into highly toxic, bifunctional adducts (48). Therefore, inhibition of topo II could enhance interstrand cross-links and CDDP toxicity, as described in previous reports (37, 38).

In conclusion, cisplatin-resistant L1210/10 cells have an increased topo II activity, which is the consequence of a qualitative modification of the enzyme. This modification contributes to the resistant phenotype of these cells.

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