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Enhanced DNA Cross-link Removal: The Apparent Mechanism of Resistance in a Clinically Relevant Melphalan-Resistant Human Breast Cancer Cell Line

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SUMMARY

Resistance to the cytotoxic effects of alkylating agents is a major limitation to their clinical efficacy. Although a number of animal and human tumor cell models have been developed to study this problem, it has proven difficult to achieve very high levels of resistance to alkylating agents *in vitro*. This is consistent with the recent clinical evidence that alkylator resistance can be overcome by dose escalations of less than 10-fold. A number of mechanisms of alkylator resistance have been described, more than one of which may occur in the same model. This paper describes a human breast cancer cell subline selected for 3-fold resistance to melphalan and cross-resistant to other alkylators in which only one of the previously described mechanisms of resistance, enhanced removal of DNA interstrand cross-linking, is demonstrable. Northern blot analysis using the human incisional repair gene *ERCC-1* cDNA demonstrated that this particular gene product is not the altered function in these cells, so the molecular characterization of the observed enhanced repair is pending. Because these cells are also cross-resistant to radiation and to adriamycin and epipodophyllotoxin, they may represent a clinically relevant model in which to examine the role of DNA repair of lesions resulting from alkylators and other cytotoxic agents.

Although antineoplastic drugs capable of alkylating DNA are clinically very active, their therapeutic potential is limited by the development, by tumors, of resistance to their cytotoxic effects. It is generally considered that the cytotoxicity of bifunctional alkylators relates to DNA cross-link formation quantitatively (1). A useful model to study mechanisms of actions of therapeutic agents has been the examination of drug-resistant mutants (2–4), and sublines resistant to a variety of drugs have been established. In the case of alkylators, it has been found that the magnitude of resistance that can be achieved is limited. This is consistent with recent clinical evidence, which suggests that resistance that occurs in patients treated with these agents is of the order of 2–10-fold because increasing the dose above this range appears to overcome the resistance (5). Cell lines selected for greater than 10-fold resistance to alkylators or other agents, including those with the phenotype of multi-drug resistance to "natural product" antineoplastics, generally have a number of concurrently demonstrable mechanisms of resistance (6). This paper describes a human breast cancer cell line selected for 3-fold resistance to MLN, in which the apparent mechanism of resistance to alkylating agents is enhanced DNA repair. These cells are cross-resistant to a broad spectrum of alkylating agents, as well as to adriamycin and radiation, which implies other concurrent mechanisms of resistance. They may be of particular relevance to the study of drug resistance observed in the clinical setting.

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Experimental Procedures

Materials

Unlabeled MLN and [chloroethyl-^{14}C]MLN (10.9 mCi/mmol) were kindly supplied by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute (Silver Spring, MD) (NSC No. 8806). Unlabeled MLN solutions were prepared daily in 75% ethyl alcohol containing an equimolar concentration of hydrochloric acid. Further dilutions were made in aqueous medium immediately before use, to minimize hydrolysis. Radiolabeled MLN was dissolved in absolute ethanol and stored at −20°. Thin layer chromatography revealed that the drug was stable for weeks at this temperature.

Bovine serum albumin was obtained as serum fraction V from Miles Laboratories (Elkhart, IN). Phosphate-buffered saline (Dulbecco's modification), RPMI 1640 medium, and trypsin-EDTA were purchased from GIBCO Canada (Burlington, Ontario). The Versilube F-50 silicone oil (specific gravity, 1.045 at 25°; viscosity, 70 centistokes at 25°) was purchased from Harwick Chemical Corporation (Cambridge, MA).

ABBREVIATIONS: MLN, melphalan; BSO, butathione sulfoximine; EB, ethidium bromide; SDS, sodium dodecyl sulfate; MOPS, 3-[*N*-morpholino] propanesulfonic acid; WT, wild-type; SSC, standard saline citrate; GST, glutathione-*S*-transferase.

BSO was obtained from Sigma Chemical Company (St. Louis, MO). PAG is amino acid-free medium composed of phosphate-buffered saline, pH 7.4, with 0.1 mM bovine serum albumin and 0.25% glucose.

Cells

MCF-7 cells are derived from a patient with previously untreated breast cancer. Both WT and resistant cells were grown in RPMI 1640 that contained 5% fetal bovine serum, 2 mM glutamine, and antibiotics. Cells are passaged weekly using trypsin-EDTA. MLN-resistant cells (MLN^R) were selected by repeated exposure of surviving cells to the drug at 2-weekly intervals with slowly escalating doses. Melphalan was dissolved in acid alcohol for use each time. Over the course of 8 months, MLN concentration was increased from 1 to 5 μM. Clonogenic assays were performed by plating cells at a density of 2–400/well in 6-well Linbro flasks (Fisher Scientific, Montreal, Quebec) and allowing them to attach to the flasks overnight. The following day, the drug was added to the medium and left for the duration of the growth period (10–14 days). For internal consistency, the clonogenic assays were repeated with the identical conditions used in the drug accumulation and cross-link studies. In these studies, cells were exposed to various doses of freshly prepared MLN in PAG medium for 35 min only, and then fresh drug-free medium was replaced. For the experiments with BSO, BSO was added to the medium 4–5 hr before the addition of MLN. Both MLN and BSO were left in the medium for 10–14 days, after which the medium was removed and cells were fixed and stained with methanol/acetic acid and Coomassie blue. Colonies in untreated wells were counted as controls, and the colonies in the treated wells are expressed as percentage of control. Each dose level was done in triplicate. For radiologic studies, cells in exponential growth were irradiated with ^{60}C γ rays and then plated as above. The dose rate was approximately 1.2 Gy/min. Colonies were fixed and counted as above.

Drug Accumulation and Metabolism

Cells at a concentration of 6×10^6 cells/ml in PAG were preincubated for 15 min at 37° before addition of [*chloroethyl*-^{14}C]MLN (5.4 μM). At 1, 3, 9, and 35 min and 3 hr after addition of [^{14}C]MLN, 400 μl aliquots of the incubation mixture were layered onto 1 ml of Versilube F-50 silicone oil in microcentrifuge tubes and then centrifuged at 12,000 × *g* for 1 min at room temperature. The resulting cell pellets were resuspended in 0.2 N NaOH, kept overnight at 4°, and then neutralized with an equivalent volume of 1 N HCl. After the addition of 10 ml of liquid scintillation cocktail, the samples were counted using a 1217 Rackbeta counter. Nonspecific absorption of labeled drug was determined by layering 200 μl of cell suspension onto 200 μl of medium that contained labeled MLN and then centrifuging as described above. The intracellular water space was determined as described previously (7). There was no difference between WT and MLN^R cells. Drug accumulation was thus calculated as the cell-to-medium ratio of drug.

Duplicate samples of the 35-min and 3-hr incubation mixtures were suspended in 70% ethanol/30% 1 M acetate buffer, pH 4.0, and stored in liquid nitrogen. After thawing, supernatants were separated by centrifugation at 12,000 × *g* for 3 min and were analyzed for intact MLN by thin layer chromatography on 13255 cellulose plates (Kodak) using isopropyl alcohol/formic acid/water (65:1:24) solvent, as described previously (8). Using this method, chromatograms identical to those presented previously in L1210 mouse leukemia cells were generated (8). As in the latter report, mono- and dihydroxy-MLN could not be distinguished and only the principle metabolite, dihydroxy-MLN is reported. Intact MLN had an R_f of 0.88, and dihydroxy-MLN had an R_f of 0.58. The recovery of radioactivity was calculated as a percentage of total radioactivity for each compound.

Biochemical assays

GSH was assayed according to the technique of Tietze (9). At least 5×10^6 cells were spun down to a pellet and then resuspended in 3% sulfosalicylic acid. After centrifugation at 10,000 × *g* for 2 min, the supernatants were used to measure GSH. Results are expressed as nmol/10^6 cells.

GST was assayed in cytosolic preparations of cells. After lysis by vortexing in H_2O, the cells were homogenized and centrifuged at 100,000 × *g* for 1 hr. The resulting supernatant was used to assay GST with 2,4-chlorodinitrobenzene as substrate (6).

Detection of DNA Cross-links

The tumor cells, at 2×10^6 cells/ml, were incubated at 37° with unlabeled MLN or vehicle for 35 min in PAG. The cell suspensions were then washed once with PAG and resuspended in an equivalent volume of PAG for a 4-hr incubation at 37° to allow for development of DNA cross-links, as previously described (10). Four hours was chosen on the basis of the experimental findings of Ross *et al.* (10). In their studies, MLN-induced DNA cross-links formed very rapidly during the first 4 hr after drug treatment, reaching approximately 85% of the peak number formed. The remaining cross-links formed more slowly during the next 2 hr. A subsequent study with the addition of proteinase K to inhibit DNA-protein cross-links demonstrated similar kinetics (11). Cross-link formation in each cell line was examined at 2.5–10 μM MLN. To examine removal of cross-links, cells were exposed to 10 μM MLN for 35 min and then incubated in drug-free PAG for 4 and 24 hr. In the previously cited study, cross-links induced by MLN were slowly removed in L1210 cells, so that at 24 hr a significant amount of cross-linked DNA remains. Cell viability was assessed by trypan blue exclusion and greater than 95% of cells were viable by this assay at the end of the incubation. The limited toxicity at this dose in this assay, compared with the clonogenic assay, is probably due to the shorter drug exposure time as well as the greater cell number treated.

Cross-linking of DNA by MLN was detected by utilizing an EB fluorescence assay, as previously described (12, 13). Forty microliters of the cell suspension (7×10^5 cells) were added to 200 μl of a lysing solution [4 M NaCl, 50 mM KH_2PO_4, 10 mM EDTA, and 0.1% (w/v) sarkosyl (pH 7.2)]. Twenty microliters of heat-inactivated bovine pancreas RNase (2 mg/ml) were added to the lysates, which were then incubated at 37° for 16 hr. After the incubation, 25 μl of heparin (500 IU/ml) were added to 3 ml of a solution that contained EB (10 μg/ml), 20 mM K_2HPO_4, and 0.2 mM EDTA (pH 12.0). The EB solution was placed in test tubes that were wrapped in aluminum foil to prevent light-induced cleavage of DNA by EB. The DNA in the resulting lysates was denatured by heating at 100° for 5 min and was rapidly cooled to 22°. Fluorescence was measured in 1-cm^3 cuvettes at 22° in a SPF-500C SLM-Aminco spectrofluorometer. The excitation wavelength was 525 nm and the emission wavelength was 580 nm. Fluorescence was measured before and after denaturation. The percentage of cross-linked DNA was calculated from measurements of the difference in fluorescence of denatured control cell lysates and the denatured MLN-treated samples by the formula:

$$C_+ = \frac{F_+ - F_n}{1 - F_n} \times 100\%$$

where C_+ = percentage of interstrand cross-linked DNA in treated cells; F_+ = fluorescence after denaturation divided by fluorescence before denaturation of treated cells; and F_n = fluorescence after denaturation divided by fluorescence before denaturation of control cells. This technique measures DNA-DNA and not DNA-protein cross-links. The substitution of proteinase K for heparin does not alter cross-link measurement using this assay (14).

Northern Blot Analysis

DNA probes. The *SalI*/*Eco*RI insert of plasmid pGP5 was used to detect the Yp subunit of GXT and was the kind gift of M. Muramatsu, Tokyo University (15). The *Pst*I insert of plasmid pGTB38, which hybridizes to both the Ya and Yc GST subunits, and the *Pst*I insert of pGTA44, which hybridizes to the Yb subunit, were the kind gifts of C. Pickett, Merck-Frosst, Montreal, and were used to probe for these respective RNAs (16, 17). The MDR1 probe was provided by P. Gros, McGill University, Montreal, and hybridizes to a gene that is overexpressed in many cell lines that are resistant to adriamycin (18). The

probe used to detect the *ERCC-1* gene is the *Eco*RI/*Pvu*II digest of plasmid pE12-12, kindly provided by van Duin, Erasmus University, Rotterdam) (19). This human gene complements a Chinese hamster ovary mutant cell line that is deficient in repair of DNA damage induced by UV irradiation and also the cross-linker mitomycin C. The cDNAs were radiolabeled with [^{32}P]dCTP (ICN Radiochemicals, Irvine, CA) to high specific activities, using the oligolabeling kit from Pharmacia (Dorval, Quebec).

RNA isolation and Northern blots. Total cellular RNA was isolated using a modification of the procedure of Chirgwin *et al.* (20). Cells in 100-mm tissue culture plates were washed once with ice-cold phosphate-buffered saline and were then lysed in a 2-ml solution containing 4 M guanidine isothiocyanate, pH 7.0, 25 mM sodium citrate, 0.1 M mercaptoethanol, and 0.5% sarkosyl. The cells were scraped off the plates with a Teflon policeman and, after a brief and gentle vortex, the lysate was layered onto 1.3 ml of a 5.7 M cesium chloride solution containing 25 mM sodium acetate. This was centrifuged at 32,000 rpm at room temperature for 20 hr. The gellatinous pellet was dissolved in 200 μl of diethylpyrocarbonate-treated water containing 0.3 M sodium acetate, precipitated in ethanol, and finally redissolved in diethylpyrocarbonate-treated water. RNA was quantitated by measuring the absorbance at 260 nm. Twenty microliters of RNA were denatured at 65° for 10 min in a solution containing 20 mM MOPS, 50% formamide, and 6% formaldehyde and were then resolved electrophoretically in a 1% agarose gel containing 0.66 M formaldehyde, using a running buffer containing 20 mM MOPS, 0.5 mM sodium acetate, and 1 mM EDTA, pH 7.0. The RNA was blotted onto a Hyband-N membrane (Amersham) with 20× SSC. The air-dried membrane was briefly exposed to UV light to immobilize the RNA on the membrane.

Hybridization was done with a modification of the method of Church and Gilbert (21). Prehybridization was performed, at 42° for 1–2 hr, in a solution containing 0.5 M $NaHPO_4$, pH 7.2, 5% bovine serum albumin, 1 mM EDTA, and 5% SDS. Hybridization was done in the same solution at 42° for 20–24 hr. After hybridization, the membrane was washed at room temperature with multiple changes of 2× SSC solution containing 0.1% SDS and at 65° with 0.2× SSC solution with 0.1% SDS. The membrane was then rinsed in 1× SSC, blotted dry, and exposed for 24–48 hr at −70° to XAR-5 Kodak X-ray film.

Results

Cell biology. MLN^R cells were found to have a longer doubling time, compared with the WT cells (24 versus 17 hr), consistent with many other published drug-resistant sublines. Fig. 1 shows the drug sensitivity of MLN^R compared with WT MCF-7 cells seen in the clonogenic assays done with the drug added and left in the medium. The MLN^R cells are approximately 3-fold resistant to MLN. This is stable for up to 5 months in drug-free medium, before cells begin to revert to the sensitivity of WT cells. For the drug sensitivity assay done with 35-min exposure in PAG medium, the IC_{50} values are shifted only about 2-fold to the right. This is consistent with the very short (less than 2 hr) half-life of MLN *in vitro* in a variety of solutions (22). Table 1 shows the cross-resistance pattern of MLN^R cells to other alkylating agents. Greatest resistance is shown to nitrogen mustard (8-fold), but in addition there is some degree of resistance (2–3-fold) to nitrosourea, cisplatin, Adriamycin, and VP-16.

The radiation response curve of MLN^R versus WT cells is shown in Fig. 2. There is no significant change in intrinsic radiosensitivity, manifest as the exponential slope of the survival curves (WT $-D_o$ = 139 ± 3; MLN^R $-D_o$ = 152 ± 7). However, a shoulder, representing sublethal damage accumulation and potential for repair, is apparent in the MLN^R cells. The extrapolation number (n) for the MLN^R cell survival curve is 2.24 ± 0.23, whereas that of the WT cells, with no apparent shoulder, is 0.9 ± 0.09.

Continuous exposure of both cell lines to BSO over 10–14 days could not be achieved at more than 4 μM concentration without significant cytotoxicity from BSO alone. At 4 μM BSO, GSH levels are diminished by approximately 25% at 4 hr and progressively decreases with time (data not shown). There is enhancement of the cytotoxicity of MLN in both the WT and MLN^R cells by 1.5–3-fold, with continuous exposure to BSO. On the other hand, pretreatment with 20 μM BSO for 20 hr before addition of MLN, which reduces GSH by 75% in both WT and MLN^R cells, had no effect on sensitivity to MLN unless there was maintenance of BSO in the medium. BSO treatment does not affect GST activity. Pretreatment with VP-16 does not sensitize cells to MLN, in contrast to the prediction of a previous report on other alkylator-resistant cells (23).

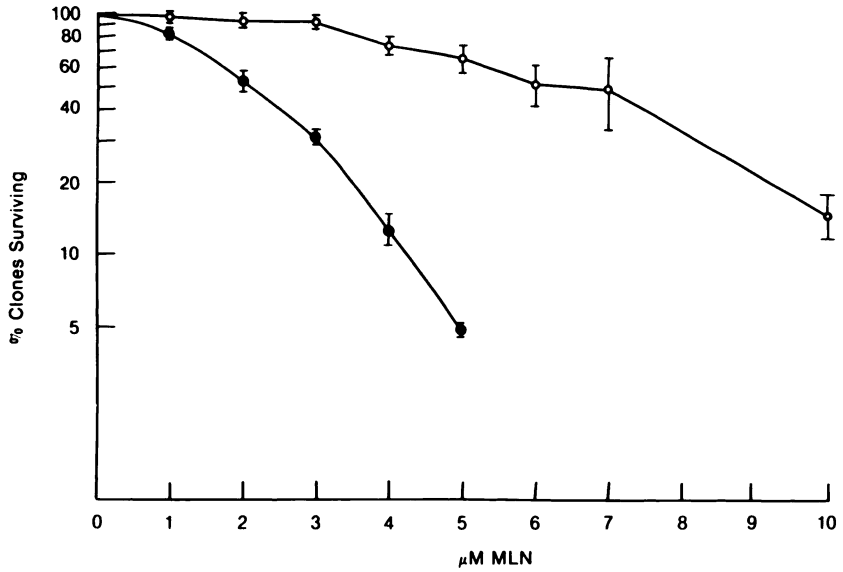

Fig. 1. The results of six separate clonogenic assays performed with the WT (●) and MLN^R (○) cells. Four hundred cells were plated per well and, after adhering, were exposed to various doses of MLN. After 10–14 days, the cells were fixed and the colonies were counted. The *points* are the mean values at each dose and the *bars* are standard errors.

TABLE 1

Cross-resistance of MLN^R compared with WT cells

Clonogenic assays were performed as described in Experimental Procedures. Low concentrations of cells were plated and then exposed to various doses of the drugs listed in the table. Numbers given are IC_{50}, the concentration of drug resulting in 50% colony survival, in μM of drug. These represent the results of at least two experiments with each drug.

Drug	IC_{50}		Fold resistant
	WT	MLN^R	
	μM		
Melphalan	2.1	6.5	3
Mustargen	0.95	7.9	8
bis-chloronitrosourea	11	26	2.4
Cisplatin	1.2	2.6	2
Mitomycin C	0.0078	0.0137	1.8
Adriamycin	0.02	0.05	2.5
VP-16	0.095	0.24	2.5

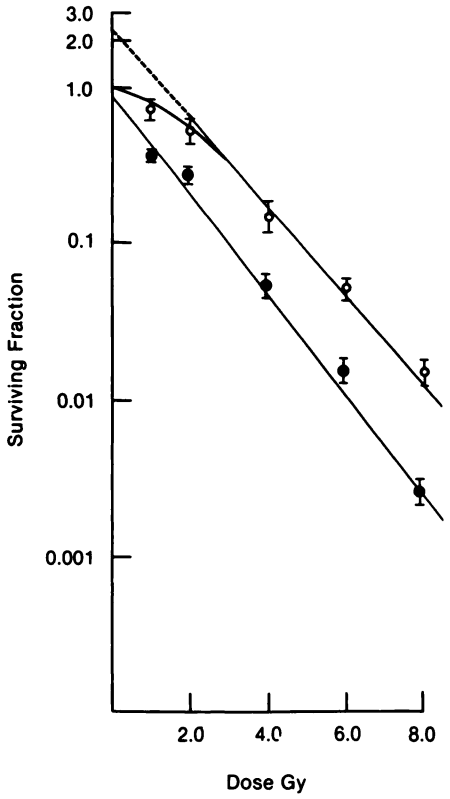

Fig. 2. The radiation sensitivity curve of the WT (●) and MLN^R (○) cells exposed to various doses of ^{60}C γ irradiation. Exponentially growing cells were treated, plated, and subsequently counted as in the drug assays. Results here are mean ± standard error of four different experiments.

Cytogenetic analysis demonstrated no homogeneous staining regions or double minute chromosomes to suggest gene amplification.

Drug transport and metabolism. Figure 3 demonstrates cellular accumulation of [^{14}C]MLN in the WT and MLN^R cell line. As can be seen, there is no difference in the peak cell to medium ratio achieved in the two cell lines (30.1 ± 3.4 versus 30.7 ± 3.2).

Biochemistry. The GSH levels in the WT and MLN^R cells were 15.9 ± 1.5 and 19.9 ± 1.6 nmol/10^6 cells (mean ± SE), respectively. Although slightly higher in MLN^R cell lines, there is no significant difference. The GST activity with 2,4-chlorodinitrobenzene as substrate was 7.3 ± 1.5 nmol/min/mg of protein for WT and 7.1 ± .8 nmol/min/mg of protein for MLN^R cells, also not significantly different.

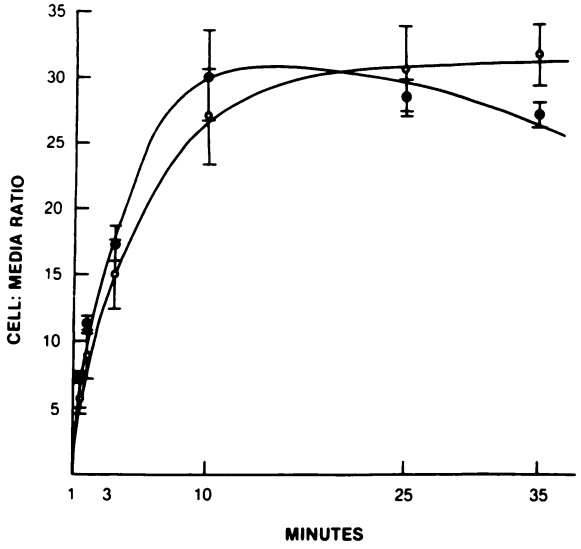

Fig. 3. The cell-to-medium ratio of radiolabeled MLN in the WT (●) and MLN^R (○) cells, at different time points (mean ± standard error). Cells were incubated with radiolabeled MLN and then collected by centrifugation. The cell-to-medium ratio was determined.

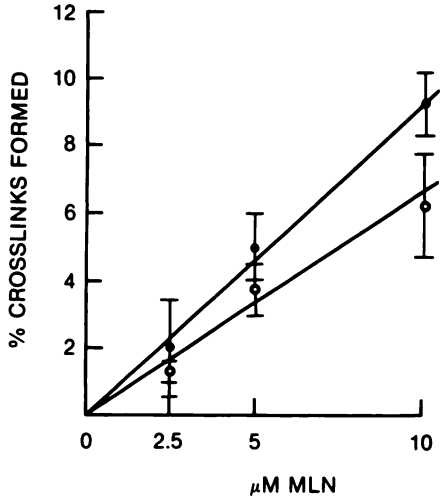

Fig. 4. The $\%C_+$ in WT (●) and MLN^R (○) cells exposed for 35 min to different doses of MLN and then maintained in drug-free medium for 4 hr to allow formation of cross-links. These were measured using the ethidium bromide assay, as described in Experimental Procedures.

Metabolism. Chromatographic analysis of the WT and MLN^R cell preparations after a 35-min incubation with 5.4 μM radiolabeled MLN revealed no evidence of metabolism to dihydroxy-MLN. The total recovery of radiolabel as intact drug was approximately 70% in both cell lines. After a 3-hr incubation, there was some metabolism but there was no significant difference in the percentage of dihydroxy-MLN present in the WT compared with the MLN^R cell preparations (25% versus 20%).

DNA cross-link formation and removal. Fig. 4 demonstrates the cross-links formed after 4 hr of incubation in drug-free medium, which followed the 35-min drug exposure. Results are expressed as percentage of interstrand cross-linked DNA ($\%C_+$), measured after cell exposure to various doses of MLN. Results are mean ± standard error of three experiments in each

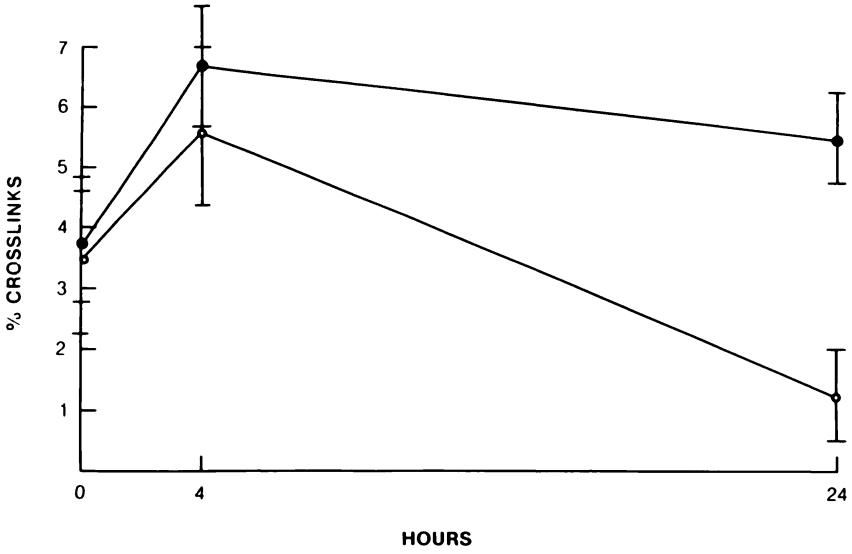

Fig. 5. The $\%C_+$ of WT (●) and MLN^R (○) cells detected at 4 and 24 hr after a 35-min exposure to 10 μM MLN (mean ± standard error). The cells were exposed to MLN for 35 min and then transferred to drug-free PAG medium. Duplicate samples were harvested after 4 and 24 hr for assay of cross-links.

cell line. Although there is a trend towards decreased cross-links in the MLN^R cells, in contrast to previously described alkylator-resistant cell lines, there is no significant difference at any of these doses. On the other hand, Fig. 5 demonstrates the $\%C_+$ at 4 and 24 hr after a 35-min exposure to 10 μM MLN. The results are the mean ± standard error of six experiments with WT cells and five experiments with MLN^R cells. Although there is no difference at 4 hr, at 24 hr there is a statistically significant decrease in the MLN^R cells compared with the WT cells (1.3 ± 0.8% versus 5.5 ± 0.7%; $p = 0.002$).

Northern blotting. Northern blot analysis did not demonstrate a difference in the concentration of specific mRNAs expressed for GST subunits, and MDR1 mRNA baseline expression observed in the WT cells was not increased in MLN^R cells (data not shown). The ERCC-1 probe hybridized to a 1.1-kilobase mRNA sequence in both WT and MLN^R cells, and there was no difference between them that could be determined by densitometric analysis (Fig. 6).

Discussion

The antineoplastic drugs that alkylate DNA represent a clinically extremely effective class of agents. Virtually all modern multi-drug treatment regimes include at least one of them. One of the principle factors limiting the effectiveness of alkylating agents is tumor resistance. There is considerable evidence that the cytotoxicity of these drugs results directly from DNA cross-linking (inter- and intrastrand). The mechanism of resistance in clinical specimens is not yet established, but there are a number of *in vitro* models in both human and animal tumor cell lines (2–4, 24). These include altered cellular drug accumulation and metabolism or detoxification. Metabolism of bifunctional alkylators may include GSH conjugation of the drug or of its metabolite, or alternatively, GSH conjugation of alkylator-DNA monoadducts (25). In a number of murine and human resistent cell lines, depletion of cellular GSH results in sensitization to the cytotoxicity of MLN (26). BSO specifically inhibits the rate-limiting GSH synthetic enzyme. The fact that GSH depletion sensitizes both WT and MLN^R cell lines has been previously reported (14) and suggests that, although GSH plays a role in the cellular response to DNA alkylators, it does not represent the difference between sensitive and resistant cell lines. A potential role for GSH in cellular repair in both WT and MLN^R cells was suggested in studies of DNA strand breaks induced by irradiation. There was a protective effect of GSH augmentation in cells after exposure to X-rays (27), similar to the effects seen of modulation of cellular GSH on human fibroblast repair of X-ray-induced DNA strand breaks (28). There is *in vitro* evidence that GSH conjugation can be catalyzed by a specific cationic isoenzyme of GST, and murine tumor cell lines selected for resistance in chlorambucil and in MLN have been described that have increased levels of specific GST isoenzymes (10, 13, 29). Decreased DNA cross-linking found in resistant cells could result from the above mechanisms or, alternately, from an enhanced rate of cross-link removal, as was described in studies of a murine tumor cell line (30. Enhanced DNA repair activity, which was measured as unscheduled DNA synthesis, has already been shown in human ovarian cancer cell lines selected for resistance to cisplatin and MLN (31).

Among the *in vitro* models used to study alkylator resistance, the level of resistance varies between 8- and 30-fold. Yet recent clinical evidence, using autologous bone marrow transplantation to allow dose escalation, suggests that, in patients treated with alkylating agents, clinical resistance is in the range of 2–10-fold because dose increases of this magnitude result in tumor responses (5). The MLN^R human breast cancer cell line described here may represent a particularly relevant model. The only mechanism of resistance that could be established is enhanced or accelerated DNA excision repair. We suggest that this may be an important mechanism of resistance in the clinical situation. We also found that, although the basal GSH levels are not different in the MLN^R cells, depletion of GSH results in sensitization of both WT and MLN^R cells.

The cross-resistance pattern of these cells is broad and even includes radiation and natural product antineoplastics, most of which cause DNA strand breaks but not cross-linking. A similar pattern of cross-resistance was previously described in a human ovarian cancer cell line resistant to MLN (16). Also, a recently described *in vivo* model of sarcoma sublines resistant to either vincristine or L-phenylalanine mustard demonstrates cross-resistance between these two classes of drugs (32). The mechanism of this cross-resistance is not evident, but these systems

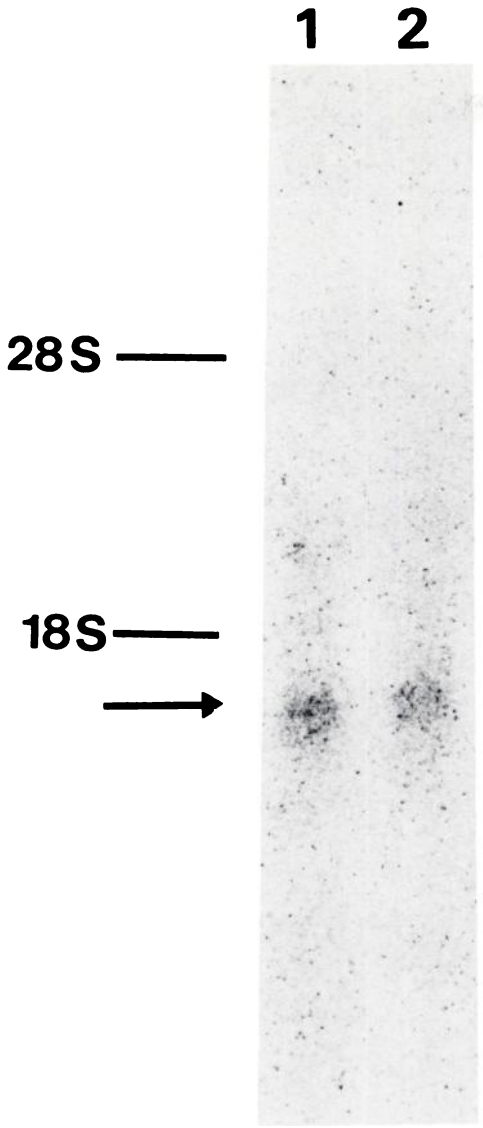

Fig. 6. The results of Northern blot analysis of total RNA from WT (*lane 1*) and MLN^R (*lane 2*) cell lines. After electrophoresis, the RNA was transferred to a Hybond-N membrane and hybridized with a cDNA complementary to the *ERCC-1* gene RNA transcript of 1.1 kilobases (*arrow*). The positions of the 18S and 28S ribosomal markers are shown.

reflect the clinical observation of cross-resistance between alkylators and natural product antineoplastics. Our model suggests either common mechanisms of DNA repair of lesions caused by these different agents or co-regulation of different DNA repair mechanisms in these cells. A relation between radiation sensitivity (DNA strand breaks) and cisplatin sensitivity (DNA cross-link formation) has been recently demonstrated in a number of human tumor cell lines (33). The recent cloning of human DNA sequences encoding a number of repair enzymes may provide useful probes to examine clinical specimens for evidence of this mechanism (34). One such human gene, *ERCC-1*, which is involved in the incision step of repair of DNA abnormalities, was shown in this study to apparently not play a role in the resistance observed in the MLN^R cells. Complementation studies using DNA from these cells and repair-deficient recipient mutants may help define the molecular basis of resistance in these cells.

Acknowledgments

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