Differential Repair of 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea-induced DNA Damage in Two Human Colon Tumor Cell Lines

Leonard C. Erickson,1 Rainhardt Osieka,2 and Kurt W. Kohn

Laboratories of Molecular Pharmacology [L. C. E., K. W. K.] and Chemical Pharmacology [R. O. J., Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Maryland 20014

ABSTRACT

Two human colon tumor cell lines were examined for their responses to 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea treatment when maintained as cultured cell lines and xenograft tumors in nude mice. One tumor line, HT, was resistant to 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea treatment both in tissue culture and in vivo. The other tumor line, BE, was sensitive to 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea treatment in vitro and in vivo. The DNA of tissue-cultured cells treated with 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea was examined by alkaline elution for DNA damage. 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea was found to produce DNA strand breaks and DNA cross-links in both cell types. The DNA cross-links appear to be completely repaired in the resistant HT line over the 48-hr period following drug removal, but in the sensitive BE line little or no cross-link repair was observed during this interval.

INTRODUCTION

For nearly 20 years the primary chemotherapy treatment for cancers of the colon and rectum has been 5-fluorouracil (NSC 19893), yielding response rates of approximately 20% with little increase in patient survival (3, 16, 17, 19). Since initial clinical trials (23), MeCCNU5 has been used in the treatment of a variety of cancers including colorectal cancer. MeCCNU treatment of advanced gastrointestinal adenocarcinomas has been reported to give response rates equivalent to those obtained with 5-fluorouracil (15). Despite some successes in treatment of this disease, many colorectal tumors show little or no response to drug therapy. Experiments with model systems may provide insights into the molecular actions of antitumor drugs on tumor cells and into the relationship of these effects to patterns of drug resistance and sensitivity.

At the cellular level MeCCNU has been shown to prolong the DNA-synthetic phase of the cell cycle in normal and leukemic mouse cells in vivo and to depress DNA synthesis in normal and malignant tissues of mice treated with MeCCNU (1). In synchronized human lymphoma cells, MeCCNU was most effective in killing cells during early S phase (4). However, in another study MeCCNU was more toxic to noncycling Chinese hamster cells than to cycling cells (20).

Several recent reports from this laboratory have demonstrated that the 1-(2-chloroethyl)-1-nitrosoureas are capable of producing DNA interstrand cross-links. 1-(2-Chloroethyl)-1-nitrosourea (NSC 47547), BCNU, and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (NSC 79037) were shown to produce cross-links in purified DNA reacted with the drugs in vitro (13). The proposed mechanism of cross-link formation involved chloroethylation of a nucleophilic site on 1 DNA strand, followed by displacement of a CI by a nucleophilic site on the opposite DNA strand (13). With the technique of alkaline elution (6), DNA cross-linking has been observed in L1210 cells treated with BCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, and 1-(2-fluoroethyl)-3-cyclohexyl-1-nitrosourea (NSC 87974), and cross-linking has also been reported in normal and transformed human fibroblasts exposed to BCNU (5). Although MeCCNU was not included in any of the studies mentioned above, it would be expected that upon decomposition this drug would also generate a chloroethyl-alkylating moiety (21), which would be capable of cross-linking DNA.

In a recent report by Osieka et al. (18), experiments were described in which human colon tumors maintained as xenografts in nude mice were treated with a variety of antitumor compounds. The only "cure" was observed with MeCCNU, which elicited a complete response in one tumor (BE) and showed no effect on another tumor (HT). In this study the DNA damage produced by MeCCNU in cultured cell lines of these 2 tumors has been examined. We have attempted to determine whether differences exist in either the induction or repair of DNA damage in the sensitive and resistant tumor cells. These experiments demonstrate that MeCCNU produces DNA strand breaks and DNA cross-links in both tumor cell lines. However, in the sensitive line (BE), repair of the DNA cross-links appears to be significantly depressed.

MATERIALS AND METHODS

Cell Culture. Cell line BE was obtained from Dr. B. Giovanela, St. Joseph's Hospital Cancer Research Laboratory, Houston, Texas. Cell line HT (HT-29), originally isolated by Fogh and Trempe (7), was obtained from Dr. E. Jensen, Mason Research Institute, Rockville, Md. Both cell cultures were grown in Eagle's MEM supplemented with 15% fetal calf serum (Flow Laboratories, Rockville, Md.), NaHCO3 (2.0 g/liter); 3% glutamine (2.3 ml/liter), and gentamicin (50 µg/ml; Schering Corp., Kenilworth, N. J.), and...
were seeded at weekly intervals and contained 2.5 x 10^5 cells in 10 ml MEM. For DNA assays, [2-14C]thymidine (0.02 mCi/ml; specific activity, 20 Ci/mmmole; New England Nuclear, Boston, Mass.) was added to cultures for 24 hr and was replaced with nonradioactive medium for an additional 24 hr. Cells were harvested by 1 of 2 methods: (a) for dose-response experiments, cells were harvested by rinsing the culture with 5 ml Hanks' BSS containing 0.02% EDTA, by treating with 1 ml 0.05% trypsin (Grand Island Biological Co., Grand Island, N. Y.) solution for 3 to 5 min at 37° and by resuspending in 4 ml MEM; (b) for alkaline elution experiments, cells were harvested by rinsing the cells with BSS containing 0.02% EDTA, by scraping the cells with a rubber policeman into 1 ml BSS, and by resuspending in 4 ml MEM. Cell number was determined with a Model B Coulter Counter (Coulter Electronics, Hialeah, Fla.).

**Animals and Xenograft Tumors.** Experiments with the human colon tumor xenograft material and the nude mouse system have been described in detail (18). Nude mice (NIH Swiss random bred) were obtained from the Frederick Cancer Research Center, Frederick, Md., and were maintained according to committee recommendations for the care of nude mice (2). Tumor BE was obtained from Dr. B. Giovannella as an established nude mouse xenograft. HT xenograft tumors were developed by s.c. injection of 10^7 tissue-cultured HT-29 cells into animals. The tumors were maintained by serial s.c. transplants. Tumor volumes were estimated at 4-day intervals from caliper measurements, and the volume was computed by the formula: length x (width)^2 x 0.5. At the time of drug treatment tumors were in the range of 100 to 300 mg.

**Drug Treatment.** MeCCNU was obtained from the Drug Development Branch, National Cancer Institute, NIH. For cell culture experiments, stock drug solutions were prepared immediately before use at a concentration of 12.38 mg/ml (0.05 M) in 95% ethanol. Appropriate dilutions of the stock solution were made so that 20 μl of drug solution were added to cultures in all experiments. Addition of 20 μl 95% ethanol had no effect on cell growth or DNA integrity in control cells.

For drug treatment of xenograft material in mice, MeCCNU was dissolved in a solution containing 10% ethanol-10% Emulphor-G20-80% sterile 0.85% NaCl. The drug was administered i.p. in a volume of 0.01 ml/g body weight to a final concentration of 18 mg/kg.

**X-irradiation of Cultured Cells.** Immediately before each experiment a single glass tube of [H-labeled L1210 cells (1 x 10^6 cells/ml) was chilled in ice, irradiated with 150 R X-ray, maintained in ice until alkaline elution, and aliquoted into chilled tubes to be mixed with BE or HT cells. In this way all experimental cells were compared to a uniform internal standard ([H-labeled L1210 cells irradiated with a single exposure to 150 R). X-irradiation was delivered by 2 vertically opposed Phillips RT-250 X-ray tubes operating at 200 KeV with 0.55-mm aluminum and 0.25-mm copper filters and a dose rate of 132 R/min. Following cell harvest, BE and HT cells were chilled in ice in individual glass tubes for X-irradiation. In all experiments all of the tubes containing MeCCNU-treated BE or HT cells, or untreated BE or HT controls, were irradiated in the same X-ray beam with 300 R. In this way, day-to-day variations in X-ray output were neutralized between the 2 cell types.

**Alkaline Elution of DNA.** The alkaline elution technique has recently been described in detail (14). In the present experiments 1 to 5 x 10^6 experimental cells labeled with [14C]thymidine were mixed in 20 ml ice-cold phosphate-buffered saline (0.15 m NaCl-0.014 m KH2PO4-0.086 m K2HPO4 with 5 x 10^6 [H]thymidine-labeled L1210 cells that had been irradiated on ice with 150 R (8). The X-irradiated internal standard permits a monitoring of individual experiments for elution anomalies. Furthermore, the data obtained may be analyzed and plotted as the fraction of the experimental DNA retained on the filters versus the fraction of the internal standard retained. The relative elution rate (B) of the experimental DNA can be determined from the relationship:

\[ B = \frac{\log (0.85) - \log (0.6)}{\log (0.85) - \log (0.6)} \]

where R is the fraction of the experimental DNA retained on the filter at 0.85 and 0.60 retention of the 150-R internal standard (5). The internal standard retention points of 0.85 and 0.60 were chosen for the following reasons. In a test of drug-induced cross-links, cells are exposed to a 300-R test X-ray dose. DNA that is broken by the irradiation but is not cross-linked elutes in a normal manner. The initial elution rates of this DNA are comparable to 300-R controls that are not cross-linked. DNA that is cross-linked is retained on the filter yielding a decreased rate of elution. The subsequent elution curve is then biphasic, with 0.85 retention the approximate cutoff point for the normally eluting DNA. The manifestation of cross-linking is to reduce the slope (relative elution rate) of the late-eluting DNA. Other intercepts may be used (e.g., 0.75 and 0.60) for calculating relative elution rates. Calculation of the data in the present experiments with the use of these intercepts yielded no differences in interpretation of the data.

The cells in 20 ml ice-cold phosphate-buffered saline were deposited on a 25-mm 2.0-μm pore-size polyvinyl chloride filter (Millipore Corp., Bedford, Mass.) by use of moderate suction and were immediately lysed with 5 ml of a solution containing 2 m NaCl, 0.02 m EDTA, and 0.3% Sarkosyl (Geigy Corp., Ardsley, N. Y.), pH 10.0. The lysis solution was allowed to flow through the filter by gravity, and the resulting lysate was washed with 3 ml of 0.02 m disodium EDTA-0.04 m NaOH, pH 10.0. Tetrapropylammonium hydroxide, pH 12.1, containing 0.02 m EDTA was pumped through the filter at a rate of 0.035 ml/min, and fractions were collected at 90-min intervals. The samples were mixed with 3.3 volumes of Aquasol (New England Nuclear) containing 0.7% glacial acetic acid. Lysing solution samples and filters were processed as previously described (14). The samples were counted in a Packard 2450B liquid scintillation counter, and the data were processed and plotted by computer.

**RESULTS**

**Comparison of Xenograft Tumor Material and Cell Culture Lines.** Histological sections of tumors BE and HT are compared in Fig. 1 to the morphology of the cultured cell lines. Tumor BE has been described as an undifferentiated
adenocarcinoma of the colon with occasional giant cells, while tumor HT is a well-differentiated adenocarcinoma of the colon (18). Both tumors contain a core of necrotic cells when tumor mass exceeds approximately 100 mg.

In tissue culture the morphology of the 2 cell lines resembles their respective tumors in vivo. Line BE grows as a relatively unorganized monolayer with occasional giant cells. Line HT grows in more ordered, multilayered patterns, and to a higher cell density than does line BE [see untreated control cell number (Chart 2)].

Response of Xenograft Tumors and Cultured Cell Lines to MeCCNU. The growth of xenograft tumors BE and HT in nude mice as untreated controls or following a single treatment of MeCCNU, 18 mg/kg, is shown in Chart 1. When these tumors are treated in the nude mouse, the growth of tumor HT is relatively unaffected by the MeCCNU treatment, whereas a complete regression is seen in tumor BE following single-dose MeCCNU treatment.

Chart 2 shows the response of BE and HT cell cultures following a 1-hr exposure to 50, 100, or 200 µM MeCCNU. The points were determined from duplicate cultures treated with MeCCNU and were harvested at various times after treatment. The experiments show that line BE is sensitive to MeCCNU treatment in tissue culture, which corresponds with its response in vivo. There is a differential response to 100 µM MeCCNU between lines BE and HT. At this dose approximately 50% of the cells in the BE cultures were lost from the plate with remaining cells showing little or no proliferation capacity, while line HT showed only a slight initial inhibition of growth with little or no cell loss. The differential sensitivity did not change over the course of the study.

Production of DNA Strand Breaks and/or Alkali-labile Sites by MeCCNU. In a recent report we showed that BCNU produces DNA strand breaks, alkali-labile sites, or a combination of the 2 lesions in the DNA of normal and SV40-transformed human fibroblasts (5). In those experiments the DNA breaks were slowly repaired over the 96-hr period following drug removal. In the present experiments DNA strand breaks and/or alkali-labile sites can be observed immediately following a 1-hr exposure of BE and HT cells to 100 µM MeCCNU (Chart 3). DNA strand breaks are indicated by an increased relative elution rate of drug-treated DNA compared to untreated controls (Table 1).

Six hr after drug removal, DNA strand breaks were not observed in either cell line (Chart 4; Table 1). Although DNA strand breaks or alkali-labile sites are probably present at this time, DNA cross-linking in the cells has increased to levels that obscure any DNA breaks. This is not surprising, however, when one considers that the DNA cross-linking obscures a large number of DNA breaks generated by the 300-R X-ray test dose used in the cross-link assay.

Twenty-four hr after drug removal, DNA breaks are still
not observed in the BE cells, but in the HT line an increase in elution rate can be seen in the drug-treated cells (Chart 5; Table 1).

Forty-eight hr after MeCCNU exposure, the DNA cross-linking in the BE cells continues to mask any DNA strand breaks. However, in HT cells assayed at this time point, when cross-link repair is nearly complete, DNA strand breaks are clearly evident (Chart 6; Table 1).

Production of DNA Cross-linking by MeCCNU. DNA cross-linking by agents such as nitrogen mustard and BCNU (6) or UV (8) can be detected by alkaline elution. The assay for DNA cross-linking has been operationally defined as a decreased sensitivity of cross-linked DNA to X-ray; i.e., drug-induced DNA cross-links cause cross-linked DNA irradiated with 300 R X-ray to elute more slowly than does DNA that is not cross-linked and irradiated with 300 R.

Alkaline elution patterns of cultures of BE and HT cells analyzed immediately following a 1-hr exposure to 100 μM MeCCNU are shown in Chart 3. The treated cultures were divided following harvest, and 1 sample was X-rayed with 300 R. In this way it is possible to observe DNA strand breaks caused by MeCCNU in the unirradiated sample (see above) and MeCCNU-induced cross-links in the sample X-rayed with 300 R. Comparison of the elution curves of MeCCNU-treated cells irradiated with 300 R with the elution curves of 300-R controls shows that both cell lines demonstrate DNA cross-linking immediately after a 1-hr MeCCNU treatment.

In experiments for examination of the kinetics of DNA cross-linking by BCNU in L1210 cells (6), the cross-linking effect increased for approximately 6 hr following drug removal, and subsequent incubations showed little change in cross-linking patterns relative to the 6-hr situation. We have examined the patterns of cross-link formation in BE and HT cells 6, 24, and 48 hr after MeCCNU treatment. The alkaline elution patterns of cell lines BE and HT 6 hr after a 1-hr treatment with 100 μM MeCCNU are shown in Chart 4. In both cell lines DNA cross-linking has increased when compared to the cross-linking levels observed at 1 hr.

The experiments shown in Chart 5 demonstrate that DNA cross-linking in line BE, 24 hr after drug removal, has increased slightly when compared to the cross-linking at 6 hr. In sharp contrast is the cross-linking observed in line HT. In these cells the cross-linking has been reduced to approximately the level observed immediately after drug removal. The question was considered as to whether the observed results could be due to a difference in cell growth or cell loss between the 2 cell lines. This appears unlikely, since cell counts done following harvest of BE and HT controls, as well as MeCCNU-treated cells, showed approximately the same number of cells in all cultures.

DNA cross-linking patterns in cell lines BE and HT 48 hr after MeCCNU treatment are shown in Chart 6. Little or no repair of the cross-links has occurred in the BE cells during the 24- to 48-hr interval. However, in cell line HT, the repair of the DNA cross-links appears to be nearly complete at 48 hr.

The alkaline elution method used in these experiments can detect DNA interstrand cross-links as produced by nitrogen mustard and BCNU (6) or DNA-protein cross-links produced by an agent such as UV (8). The difference between these 2 types of cross-linking is that the latter can be removed by treating the lysate on the filter with proteinase K. We have examined the DNA cross-linking for proteinase sensitivity in lines BE and HT 6 hr following drug removal, when the cross-linking is approximately equivalent in the 2 cell lines. Proteinase had little effect on the DNA cross-linking assay in either cell line, indicating that MeCCNU is producing, for the most part, DNA interstrand cross-links in these 2 cell lines.

Table 1 summarizes the data from the alkaline elution experiments with lines BE and HT. Although the elution curves are not linear, the slope between 0.85 and 0.60 retention of the 150-R internal standard estimates the relative elution rate of the DNA (see "Materials and Methods"). DNA strand breaks can be observed as an increase in
FRACTION OF JH-LABELED DNA RETAINED ON FILTER

Chart 6. Alkaline elution patterns of BE and HT cultured cell lines following a 1-hr exposure to 100 µM MeCCNU and 48 hr incubation at 37° following drug removal. Symbols and lines are the same as in the legend to Chart 3.

Table 1

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Cell line BE</th>
<th>Cell line HT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300-R Relative elution rate</td>
<td>Relative elution rate</td>
</tr>
<tr>
<td>Untreated control</td>
<td>X-ray&lt;sup&gt;a&lt;/sup&gt; 8 - 0.32 ± 0.14&lt;sup&gt;d&lt;/sup&gt; 8 0.25 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>X-ray control</td>
<td>µM MeCCNU (1 hr) 9 + 2.12 ± 0.37 9 2.51 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>100 µM MeCCNU (1 hr)</td>
<td>4 - 0.32 ± 0.08 4 0.41 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>100 µM MeCCNU (1 hr; 6 hr incubation)</td>
<td>3 + 1.55 ± 0.15 3 1.20 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>100 µM MeCCNU (1 hr; 5 hr incubation)</td>
<td>4 0.73 ± 0.12 4 1.08 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>100 µM MeCCNU (1 hr; 24 hr incubation)</td>
<td>2 - 0.28; 0.26 2 0.37; 0.54</td>
<td></td>
</tr>
<tr>
<td>100 µM MeCCNU (1 hr; 24 hr incubation)</td>
<td>6 + 0.51 ± 0.08 6 1.41 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>100 µM MeCCNU (1 hr; 48 hr incubation)</td>
<td>2 - 0.31; 0.34 2 0.69; 0.85</td>
<td></td>
</tr>
<tr>
<td>100 µM MeCCNU (1 hr; 48 hr incubation)</td>
<td>2 + 0.60; 0.81 2 2.62; 2.81</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> n, number of independent experiments.
<sup>b</sup> X-ray was delivered to cells after completion of MeCCNU treatment; the cells were kept on ice during and after X-ray.
<sup>c</sup> See "Materials and Methods" for definition and computation of relative elution rate.
<sup>d</sup> Mean ± S. D. is used for 3 or more determinations; individual values are used for less than 3 determinations.

elution rate relative to the untreated controls, and DNA cross-linking can be determined from a decrease in elution rate relative to 300-R X-ray controls.

If the relative elution rates of the DNA cross-linking assays are examined (Table 1), it can be seen that, for line BE, cross-linking increased with time through 24 hr and remained unchanged after that time point. For line HT the relative elution rate at 6 hr indicates an increase in cross-linking, while at 24 hr cross-linking has decreased, presumably by some repair process. By 48 hr repair of the cross-links is nearly complete. Chart 7 shows the relative elution rates of the 2 cell types at various times after a 1-hr exposure to 100 µM MeCCNU. It can be seen that cross-linking increases through 6 hr in both cell types. In line BE there is little change in cross-linking levels after that time. In cell line HT, repair is clearly evident in the time interval between 6 and 48 hr.

DISCUSSION

In this report we compare the response to MeCCNU of 2 human tumor lines of colonic origin. One line, HT, is resistant to MeCCNU treatment whether it is maintained as a solid transplantable xenograft tumor in nude mice (Chart 1) or as a cultured cell line (Chart 2). The other line, BE, shows a marked sensitivity to MeCCNU when treated as a solid xenograft tumor or a cultured cell line (Charts 1 and 2). In both cell lines MeCCNU produced a significant number of DNA strand breaks when the DNA was assayed by alkaline elution following drug exposure (Chart 3). In addition both cell lines demonstrated MeCCNU-induced DNA cross-linking at 1, 6, and 24 hr (Charts 3 to 5). The observed cross-linking in line BE was relatively unchanged between 24 and 48 hr following drug removal, but in line HT the level of cross-linking decreased over the period from 6 hr after drug removal to 48 hr after drug removal (Charts 6 and 7; Table 1).

One possibility for the resistance of the HT cells to MeCCNU is that the drug was not taken up by the cells. However, the findings of DNA strand breaks and DNA cross-links in the HT cells imply, at least, that the drug reached the DNA. An alternative explanation for the resistance of the HT line, which is suggested by the data, is that these cells are capable of repairing the DNA damage produced by MeCCNU. Although cross-link repair may not be complete by 48 hr, cross-linking levels in the HT cells have been
significantly reduced in the interval between 6 and 48 hr following drug removal, whereas in the BE cells little or no repair is evident.

Several explanations for the sensitivity of line BE to MeCCNU treatment can be suggested. The first is that, unlike the HT line, the BE cells are incapable of repairing the DNA damage produced by MeCCNU. This possibility is suggested by the difference in cross-linking levels between the 2 cell lines, which exist 24 and 48 hr after drug treatment. Whereas the cross-linking in the HT cells appears to be mostly repaired, the cross-linking in the BE cells is relatively unchanged. The inability to repair the cross-links may be responsible for the cell loss observed in cell culture experiments and the regression of xenograft tumors in the nude mice. Furthermore, it is possible that MeCCNU has act on another cellular target, rendering these cells incapable of repairing the DNA damage produced by the drug. BCNU and one of its breakdown products are capable of inhibiting repair of X-ray-induced DNA damage (12). Recent experiments with normal and SV40-transformed human fibroblasts have shown that MeCCNU is also capable of inhibiting X-ray repair.* It is possible in these experiments that MeCCNU has inhibited some constituent of the cross-link repair process.

An alternative explanation is that the BE cells are dead or dying and are therefore incapable of repairing the DNA damage. However, these cells do not exhibit gross DNA degradation, which has been observed in alkaline elution experiments with severely damaged cells (K. W. Kohn, unpublished observations). To address this question, we examined BE and HT control and MeCCNU-treated cultures for their ability to exclude trypan blue. Twenty-four hr after MeCCNU removal, control and treated cultures were indistinguishable from one another when scored for trypan blue exclusion. In both BE and HT cultures, more than 99% of the cells were capable of excluding trypan blue. By this criterion the BE cultures are not exhibiting gross cell death, and the possibility that the BE cells are dead appears unlikely.

The formation and repair of DNA cross-links in sensitive and resistant Yoshida ascites sarcoma cells treated with nitrogen mustard in vivo have been reported recently (10). The resistant Yoshida cells formed and repaired cross-links in a pattern similar to that of the HT cells in this report. Similar to line BE, the sensitive Yoshida cells formed cross-links at a slower rate and showed no evidence of repair of these cross-links over a 50-hr period.

It is not known whether the induction of DNA cross-links and the inability of cells to repair cross-links are responsible for the cellular lethality of a given chemotherapy treatment. However, if the pattern of cross-link persistence exhibited by the BE cells of this report and the sensitive Yoshida cells (10) is observed with other drug treatments or cell types, monitoring these processes may provide a predictive assay for therapeutic effectiveness.

* L. C. Erickson, manuscript in preparation.

ACKNOWLEDGMENTS

The authors wish to thank Irene Clark for assisting with cell cultures, Naomi Sponsler for assisting with alkaline elution, Francis Hawkins for assisting with the nude mice, and Madie Tyler for preparing the manuscript.

REFERENCES


Fig. 1. Histological preparations of tumor BE (A) and tumor HT (B) fixed in Bouin's solution and stained with H & E. A and B, x 200. Tissue-cultured cell line BE: x 200 (C); x 320 (E). Line HT: x 200 (D); x 320 (F).
Differential Repair of
1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea-induced DNA Damage in Two Human Colon Tumor Cell Lines

Leonard C. Erickson, Rainhardt Osieka and Kurt W. Kohn

Cancer Res 1978;38:802-808.

Updated version
Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/38/3/802

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.