Auromomycin-induced DNA Damage and Repair in Human Leukemic Lymphoblasts (CCRF-CEM Cells)

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ABSTRACT

An alkaline elution procedure was used to study the nature of DNA damage induced by auromomycin, an antitumor protein, in human leukemic lymphoblasts (CCRF-CEM cells). The filter elution of drug-treated cells at pH 12.2 and 9.6 showed induction of both single and double strand DNA breaks. The DNA strand scission activities were linear in relation to drug concentration. The frequency of single strand breaks was higher than that of the double strand breaks. Protein-associated DNA single strand breaks were also detected in alkaline elution of drug-treated cells when a proteinase K digestion step was included in the assay protocol. The auromomycin-induced single strand breaks were repaired to almost completion within 8 h of postincubation of DNA-damaged cells whereas the repair of double strand breaks was not detected.

INTRODUCTION

Auromomycin, a low molecular weight protein isolated from Streptomyces macramomyceticus, exhibits in vitro and in vivo antitumor activity against a variety of experimental tumors (1, 2). DNA is the intracellular target for this antibiotic and DNA strand scission leading to inhibition of DNA synthesis is the primary event which is responsible for drug cytotoxicity (3, 4). Auromomycin is a two-component molecule consisting of a fluorescent chromophore and a M, 10,707 apoprotein (5–8). Although in itself the apoprotein appears to be inactive, its involvement on structure stability and in the biological activity of chromophore has not been established.

Earlier, in collaboration with Dr. Goldberg and his colleagues (3, 5), we have described the DNA strand scission activity of auromomycin and its chromophore by alkaline sucrose gradient analyses, in pMB and λ DNA and of DNA in intact HeLa cells. We have also, recently, confirmed (2) the DNA strand scission activity of auromomycin in human leukemic lymphoblasts (CCRF-CEM cells). In the present study, we have examined the effects of auromomycin and its chromophore on DNA of CCRF-CEM cells by the alkaline elution technique of Kohn et al. (9) in order to determine the extent and nature of DNA damage in intact cells and its repair. These studies were carried out to determine the role of DNA damage and repair in overall auromomycin cytotoxicity.

MATERIALS AND METHODS

Drugs. Auromomycin and neocarzinostatin were gifts from Kanegafuchi Industries, Ltd., Kita-ku, Osaka, Japan, and Kayaku Antibiotics, Itabashi-ku, Tokyo, Japan.

Cells. Human leukemic lymphoblastoid (CCRF-CEM) cells were grown in minimum essential medium for suspension culture (GIBCO-1385; Gibco Laboratories, Long Island, NY) supplemented with 10% fetal calf serum, penicillin (100 μg/ml), and streptomycin (100 units/ml). Cells were maintained at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% air.

 Incorporation of Radioisotopes. The DNA labeling with [3H]- or [14C]thymidine was carried out in the following manner. Cells in exponential growth phase (50 ml; 0.60–0.75 x 10^6 cells/ml) were grown in culture media containing either [methyl-3H]thymidine (1 μCi/ml; 6.7 Ci/mmol; New England Nuclear, Boston, MA) or [2-14C]thymidine (2 μCi/ml; 56 mCi/mmol; Amersham, Arlington Heights, IL). After one cell cycle (approximately 20 h) the radioactive medium was removed, and cells were washed with PBS and reincubated for 4 h in fresh medium containing 10^-6 M nonradioactive thymidine to chase the radiolabel into high molecular weight DNA. The amount of radioactivity incorporated into DNA was approximately 1.6 x 10^9 dpm for [3H] and 4.5 x 10^8 dpm for [14C] per 10^6 cells.

γ-Irradiation of Cells. Aliquots (1 ml; 0.8–1.0 x 10^6 cells) of the [3H]- and/or [14C]-labeled cells were transferred to sterile polystyrene conical tubes (110 x 116 mm; catalog no. 347886, Gibco) and chilled in an ice-water bath for 1 h prior to and during γ-irradiation. Tubes were irradiated at a distance of 25 cm from a 3400-Ci 60Co source. The dose rates used were 0–10 Gy (100 rads = 1 Gy) for the SSB assay and 0–128 Gy for the DSB assay.

Drug Exposure. 14C-Labeled cells were exposed to drugs for predetermined time and concentration at 37°C under a humidified atmosphere of 5% carbon dioxide and 95% air.

Radioactivity Measurements. The radioactivity in cell pellets was determined after two washings with PBS, precipitation with 10% trichloracetic acid, and solubilization of the trichloroacetic acid precipitate in 0.1 N NaOH. An aliquot of the alkali solution was mixed with 5 or 10 ml of Aquasol (J. T. Baker Chemical Company, Phillipsburg, NJ) for counting on a Beckman Model 700 scintillation counter.

Preparation of Auromomycin and Neocarzinostatin Chromophores. The procedures for the preparation and characterization of chromophores and apoproteins of auromomycin and neocarzinostatin are described in detail in our earlier publications (2, 8).

Alkaline and Neutral Elution of DNA Fragments. The procedures adopted by Kohn et al. (10) and Bradley and Kohn (11) were followed for alkaline (pH 12.2) and neutral (pH 9.6) filter elution, respectively, of DNA from cells after γ-irradiation or drug treatment. In these procedures elution on a 2-μm polycarbonate filter with a 0.05 M Tris-0.05 M glycine-0.02 M disodium EDTA buffer, pH 9.6, containing 2% (w/v) sodium dodecyl sulfate, determines the relative size of double strand DNA (11). The procedures for cell lysis and DNA elution were similar to those described by Kohn et al. (9). Equal number of 14C-

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labeled and ³H-labeled cells were pooled in a glass tube at ice water temperature and then added to a chilled cylinder/filter (25-mm Swinnex; Millipore Corp.) apparatus. The total number of cells per filter were 1.0–1.5 x 10⁶ for SSB and 4–5 x 10⁶ for DSB assay. The cell suspension was gravity filtered and cells were washed with 15 ml of ice cold PBS. The cells were later lysed at room temperature with 5 ml of 0.2% Sarkosyl solution, pH 10, containing 2 M NaCl and 0.04 m disodium EDTA for 20 min. The lysis solution was allowed to drain by gravity and was gently washed with 5 ml of 0.02 m disodium EDTA adjusted to pH 10. The DNA on the filter was eluted either with 0.02 m tetrahydro-EDTA adjusted to pH 12.2 with 10% tetrapropylammonium hydroxide (Eastman Kodak Co., Rochester, NY) for SSB or with Tris-glycine-EDTA sodium dodecyl sulfate buffer, pH 9.6, for DSB. The pumping rate of buffer was 0.8 ml/h. Generally, 90-min fractions were collected from each elution. At the end of elution, DNA on the tubing and filter apparatus was recovered by washing three times with 2.5 ml of 0.4 n NaOH. The DNA on the filter was solubilized with 0.4 ml of 1 n HCl at 70°C for 90 min. The eluted fractions and wash solutions, after neutralization with either 1 n HCl or 0.4 n NaOH, were mixed with sufficient Aqualyte to obtain a homogeneous phase for scintillation counting. Corrections for quenching and spillover of radioactivity during dual label counting were made according to the procedure given in the Beckman 7000 Instrumentation Manual.

In experiments in which proteinase K digestion was included in the protocol, the cell lysates retained on the filters were treated with the enzyme (0.25 mg/ml; no. 24568; E. M. Science, Gibbstown, NJ) at room temperature for 45 min in the lysing solution. The filters were later gravity drained and washed with 0.02 M disodium EDTA buffer, pH 10, and elution with buffer, pH 12.2, was continued for SSBs.

RESULTS

The alkaline filter elution analysis for quantitation of cellular DNA damage is based on molecular size-dependent retention of DNA strands on 2-μm pore size polycarbonate or polystyrene filters. In order to obtain sufficient precision for quantitation of DNA damage and concomitant repair of damaged DNA, by this methodology, we have normalized the method by: (a) determining DNA damage, SSBs, and DSBs, after exposure of CCRF-CEM cells to different levels of radiation; and (b) including 2.56-Gy γ-irradiation, we have normalized the method by: (a) determining DNA damage and concomitant repair of damaged DNA, by this method.

Effect of γ-Irradiation on CCRF-CEM Cell DNA Elution. CCRF-CEM cells were exposed to different levels of γ-irradiation at 0°C and elution of DNA from these cells at pH 12.2 and 9.6 are shown in Fig. 1. The γ-irradiation, from 0 to 7.68 Gy, produced single strand DNA lesions in cells resulting in nearly linear elution of DNA scission products through the polystyrene chloride filter up to 10 h (about 7 ml) of elution. A slight concavity in the elution pattern began to appear later. When the initial slopes of curves in Fig. 1A were plotted against different doses of radiation, the SSBs were linear in relation to radiation dose. A comparison of these data to those of Kohn's observations (9) suggested that the initial elution rate is precise and dependable for prediction of DNA damage in cells.

Alkaline elution also allows detection of DSBs when DNA was eluted from polycarbonate filters with the nondenaturing buffer at 9.6. Radiation doses approximately 10-fold higher were needed to elute DNA fragments from filters (Fig. 1B) at this pH. This is consistent with the published observations (9–11) that γ-irradiation produces one DSB for every 10–20 SSBs. When initial slopes of the curves (Fig. 1B) were plotted against radiation dosage, a linearity in DSBs, similar to the one observed for SSBs, was observed.

The next question was to determine whether the rate of elution of "test" [¹⁴C]DNA was identical to the rate of elution of "reference" [³H]DNA, both exposed to the same amount of γ-irradiation. These data were essential for our studies since 2.56-Gy γ-irradiated [³H]thymidine-labeled cells were used as an internal reference in SSB assay. In these experiments, [¹⁴C]thymidine-labeled (0.8 x 10⁶) and [³H]thymidine-labeled (0.8 x 10⁶) cells, both exposed to 2.56-Gy γ-irradiation, were mixed and placed on a filter, washed, and lysed and DNA was eluted at pH 12.2. The fraction of [¹⁴C]DNA eluted was relative to the fraction of DNA eluted from [³H]-labeled cells (data not shown) and a linear square regression analysis of the elution points of both labels gave a slope of 0.92 suggesting a linearity in the elution of the "test" and "reference" DNA. This experiment confirmed that DNA single strand products from [¹⁴C] and [³H]-labeled cells, both exposed to same amount of radiation, were similar in size and exhibited similar elution properties irrespective of the type of radiolabel associating with the DNA (19).

Effect of Auromycin on CCRF-CEM Cell DNA. Fig. 2 shows the effect of auromycin on single strand DNA breaks in CCRF-CEM cells. In these experiments [¹⁴C]thymidine-labeled cells exposed to different concentrations of drug were eluted at pH 12.2 along with [³H]thymidine-labeled cells exposed to 2.56 Gy which was used as an internal reference. The rate of elution of DNA from auromycin-treated cells were later compared to the elution rate of DNA from cells exposed to different levels of radiation (Fig. 1A) and the drug-induced DNA SSBs expressed in radiation equivalents. Fig. 2 shows that auromycin effect on DNA SSBs is drug concentration dependent and linear and that DNA SSBs by auromycin can be detected, by this assay, even at the 10 Nm drug level. The strand break frequency for auromycin, when calculated on the basis of a reported value of 0.9 DNA break per rad per 10⁶ nucleotides (9, 10), was found to be between 0.03 and 1.1 per 10⁶ nucleotides at 10-100 Nm concentrations of drug (Table 1).

Auromycin-induced DNA DSBs in CCRF-CEM Cells. The induction of double strand DNA breaks in CCRF-CEM cells after exposure to different levels of auromycin is shown in Fig. 3. In these experiments the DNA from [³H]-labeled cells exposed to
DNA DAMAGE AND REPAIR BY AUROMOMYCIN

Fig. 2. Dose dependency of DNA SSBs following treatment of CCRF-CEM cells with auromomycin for 1 h at 37°C. Elution of drug-treated [³H]thymidine-labeled CCRF-CEM cells (0.8–1.0 x 10⁸) were carried out on polycarbonate filters with pH 12.2 buffer and initial slopes of the curves were compared to the radiation-induced elution slopes to calculate for DNA damage as radiation equivalent. Equal number of ³H-labeled CCRF-CEM cells exposed to 2.56 Gy irradiation were used as an internal reference in these experiments. Each point represents the mean of ≥4 experiments. Bars, SD.

Table 1

Frequency of DNA SSBs in CCRF-CEM cells exposed to auromomycin, neocarzinostatin, and their respective chromophores and apoproteins.

Cells were exposed to drug for 1 h at 37°C. The DNA was eluted at pH 12.2 and break frequencies were calculated on the basis of 0.9 break per rad per 10⁶ nucleotides (9, 10) after converting the drug effects into radiation equivalents.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (nM)</th>
<th>DNA break frequency/10⁶ nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auromomycin</td>
<td>10–100</td>
<td>0.03–1.10</td>
</tr>
<tr>
<td>Auromomycin chromophore</td>
<td>50</td>
<td>0.32</td>
</tr>
<tr>
<td>Auromomycin apoprotein</td>
<td>200</td>
<td>0.05</td>
</tr>
<tr>
<td>Neocarzinostatin</td>
<td>50</td>
<td>0.28</td>
</tr>
<tr>
<td>Neocarzinostatin chromophore</td>
<td>200</td>
<td>0.30</td>
</tr>
<tr>
<td>Neocarzinostatin apoprotein</td>
<td>50</td>
<td>0.05</td>
</tr>
<tr>
<td>Auromomycin chromophore (50 nM) + proteinase K</td>
<td>50</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Fig. 3. Dose dependency of DNA DSBs following treatment of CCRF-CEM cells with auromomycin for 1 h at 37°C. Elution of drug-treated [³H]thymidine-labeled cells (0.4–0.5 x 10⁸) were carried out on polycarbonate filters with pH 9.6 buffer. The initial slopes of elution curves were compared to the radiation-induced elution slopes to calculate for DNA damage as radiation equivalent. Each point is the mean of ≥4 experiments. Bars, SD.

Effect of Auromomycin on Elution of DNA at pH 12.2 after Proteinase K Digestion. Proteinase K digestion of cell lysate prior to elution with pH 12.2 buffer will increase DNA elution rate and, thus, provide evidence for the existence of protein-associated DNA breaks in cells treated with intercalating agents (12–15). Since in an earlier study Kappen et al. (5) had observed that auromomycin chromophore will intercalate with DNA, the alkaline elution experiments were carried out to test whether inclusion of proteinase K digestion in SSB assay would also increase DNA elution in auromomycin-treated cells. The data obtained from these experiments are shown in Fig. 4. Without proteinase K digestion, auromomycin at 20 nM and 1 h exposure at 37°C exhibited low single strand scission activity (break frequency, 0.03 per 10⁶ nucleotides), while with proteinase K, at the same concentration, auromomycin induced a large increase in single strand scission activity (break frequency, 0.4 per 10⁶ nucleotides). This suggested that DNA strand products induced by auromomycin were probably hidden by association with a protein and prevented elution from filters. Additionally, these experiments gave evidence for the probable presence of protein-DNA cross-links. Exposure of CCRF-CEM cells to both auromomycin (20 nM) and irradiation (3.54 Gy) resulted in reduced elution of DNA fragments (break frequency, 0.26 per 10⁶ nucleotides), when compared to radiation treatment alone (break frequency, 0.35 per 10⁶ nucleotides). With proteinase K digestion, there was a complete reversal of this combined treatment effect. The

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combined effects of auromomycin and irradiation, after proteinase K digestion, increased DNA strand breaks (frequency, 0.72 per 10^6 nucleotides) and their sum effect appeared to approximate the total of individual treatments (break frequencies, 0.4 and 0.36 per 10^6 nucleotides, respectively). The data thus taken together suggested the probable existence of DNA-protein cross-links in auromomycin-treated cells.

**DNA-damaging Activities of Auromomycin, Chromophore, and Apoprotein Based on SSB Assay.** The comparative effects of auromomycin and the chromophore and apoprotein on DNA SSB in CCRF-CEM cells are given in Table 1. Their effects were compared with those of neocarzinostatin, an antibiotic protein possessing similar antitumor properties, and its components. Both auromomycin and neocarzinostatin-induced SSBs and at equimolar concentrations auromomycin appears to be slightly more active than the neocarzinostatin. The break frequencies of chromophores are similar to those of the respective native proteins. The apoproteins possessed no DNA-damaging activity and whatever little SSB frequencies seen were due to the chromophores present in the apoprotein preparations which could not be removed during their purification. Proteinase K digestion of the chromophore-treated cell lysate prior to elution resulted in a considerable increase of SSB frequencies.

**Repair of Auromomycin-induced Strand Breaks.** Fig. 5 shows the effect of postincubation, for 1, 2, 4, and 8 h at 37°C, of CCRF-CEM cells that have been previously exposed to 50 nm auromomycin. Exposure of CCRF-CEM cells to auromomycin at this concentration will result in damage of DNA as both SSB and DSBs. The postincubation of cells, at 37°C, after drug treatment resulted in the decreased elution of DNA fragments at pH 12.2, and not at pH 9.8, suggesting repair of single strand DNA breaks. The SSB repair increased linearly over a period of time and appeared to complete within 8 h. The rate of SSB repair was calculated according to the formula given by Bradley and Kohn (11).

**DISCUSSION**

Since DNA is generally accepted as the target for cytotoxicity of many antitumor drugs, studies on intracellular molecular damage induced by these agents are primarily focused on changes affecting DNA structure and function (16). Exposure of cells to DNA-interacting drugs generates several types of DNA lesions. A number of approaches and techniques have been developed to quantitate DNA damage induced by UV radiation and antitumor drugs and to monitor the repair of damaged DNA (17, 18). The alkaline sucrose gradient sedimentation and the alkaline filter elution assays have been the methods of choice. However, most DNA-damaging agents produce two or more types of DNA cuts in cells which cannot be detected by one method. The alkaline filter elution assay, which is 2–3 times more sensitive than the alkaline sucrose gradient sedimentation analysis for detection of single and double strand DNA breaks, is versatile and with this procedure one can also detect and quantitate other types of DNA damage, such as DNA-protein and DNA-interstrand cross-links and protein-associated DNA breaks.

The effects of antitumor proteins, auromomycin and neocarzinostatin, on DNA at the molecular level have been elegantly studied by Goldberg and his colleagues and several other investigators (20). Most of the DNA strand scission activities reported by these investigators are based on sucrose gradient analysis at alkaline and nondenaturing (neutral) conditions. Both neocarzin-
DNASTATIN AND AUROMOMYCIN INDUCE SINGLE AND DOUBLE STRAND CUTS IN ISOLATED DNA AND IN INTACT CELLS

The SSB with neocarzinostatin results in gaps bearing 3'- and 5'-phosphoryl termini and is associated with the release of thymine or adenine (23–27). The SSBs in isolated DNA are facilitated in the presence of mercaptans (28) and studies also suggest that a free radical mechanism (29–33) exists in the DNA cleavage event of neocarzinostatin and auromomycin. Fluorescence, circular dichroic, and biochemical studies suggest that the neocarzinostatin chromophore binds to target DNA by an intercalating mechanism (34–36). It is, however, not clear from these studies whether the double strand breaks are a direct action of the drug or a consequence of a random occurrence of two closely located SSBs on opposite strands. The mechanism and requirement for the strand scission activity of auromomycin and its chromophore are suggested to be similar to those of neocarzinostatin and its chromophore except in the requirement of sulfhydryl cofactors (3-6).

The CCRC-CEM cell line used as a model in our study is of human leukemic lymphoblastoid origin and has been extensively used for evaluation of drug sensitivity to a variety of cancer chemotherapeutic agents (37). We have earlier shown that auromomycin and its chromophore inhibit the in vitro growth of these cells by inhibiting DNA synthesis (2). DNA strand scission activity, measured by alkaline sucrose gradient analysis, was also seen in these cells which were exposed to auromomycin and its chromophore. The data obtained from alkaline filter-elution, as shown in this study, concur with our earlier alkaline sucrose sedimentation analysis in single strand scission activity. Technically, DNA elution at pH 12.2 is affected by both double and single strand breaks and the former appears as single strand breaks at this pH. Furthermore, alkaline and neutral elution analyses also show that the single and double strand breaks are drug concentration dependent. The strand scission activity of both proteins was associated with their respective chromophores, whereas the apoproteins were inactive. The interesting feature of the present study, however, is the observation on the existence of protein-associated DNA breaks in cells exposed to auromomycin and its chromophore. When a proteinase K digestion step was included in the single strand elution assay, there was an increased elution of DNA from cells exposed to auromomycin suggesting that the drug-induced strand breaks were retarded on the filter by a protein. We also noticed in these experiments a reduction in alkaline elution of DNA strands induced by 3.84-Gy irradiation of cells due to exposure of irradiated cells to 20 µM auromomycin. Proteinase K digestion reversed the auromomycin effect and, as a result of enzyme treatment, the DNA elution procedure. At the concentrations used in our experiments both single and double strand DNA breaks are possible in cells exposed to auromomycin. The data show that 80% of the single strand DNA breaks are repaired within 8 h, but when the drug-exposed and postincubated cells were analyzed by filter elution assay at pH 9.6 for repair of double strand DNA breaks, there was no repair in 8 h. The repair of drug-induced DNA breaks depends on the type of cuts inflicted on DNA and may suggest a different repair mechanism if the nicks are mediated via the involvement of topoisomerases (43). We cannot suggest any repair mechanism(s) for auromomycin-induced DNA breaks from this study.

Experiments with isolated DNA are essential to define, biochemically, the mechanisms of drug-DNA interactions leading to molecular damage. This is well exemplified in studies on neocarzinostatin and its chromophore by Goldberg and his associates and other investigators (20, 23–28, 44). The cell-free λ and pMB DNA, pLJ3 plasmid, and Zφ-HinfI long and short nucleotide fragments act as well-defined templates for accurate characterization of DNA scission products as well as understanding the mechanism of strand scission activity. The intracellular environment is lacking in vitro experimental conditions. The drug effects with intact DNA, on the other hand, take place in the complex of chromatin and replication apparatus, the physical structures and biological relevance of which are poorly characterized in mammalian cells. DNA breaks formed as a result of topological alteration of DNA by breaking-rejoining enzymes have been detected in mammalian cells and isolated cell nuclei (40, 41, 45–48). Many intercalating antitumor drugs (12–15, 38–41, 45, 46, 49) induce protein-linked DNA breaks in mammalian cells and isolated nuclei by a mechanism that is probably independent of radical formation. There has been no indication as to the probable association of a protein with the DNA cut products in studies reported hitherto on the interaction of auromomycin and neocarzinostatin or their chromophores with DNA (23–25, 33–36). This may be due to (a) strand-cutting activity being done with isolated DNA and (b) difficulty encountered in the identification of protein-associated strand breaks in alkaline sucrose density sedimentation analysis. The present alkaline elution experiments with auromomycin provide evidence for protein-associated DNA cuts in intact cells and suggest a possible involvement of enzymes similar to topoisomerase II in the target action of auromomycin. Future work should clarify the mechanism for protein-associated SSBs and protein-DNA cross-linking and correlate their involvement to auromomycin or its chromophore cytotoxicity.

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REFERENCES

2. Samy, T. S. A., Siegel, P. J., Hopper, W. E., Jr., and Krishan, A. Experimental
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