Cytotoxicity and DNA Lesions Produced by Mitomycin C and Porfiromycin in Hypoxic and Aerobic EMT6 and Chinese Hamster Ovary Cells

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ABSTRACT

Solid neoplasms may contain deficient or poorly functional vascular beds, a property that leads to the formation of hypoxic tumor cells, which form a therapeutically resistant cell population within the tumor that is difficult to eradicate by ionizing irradiation and most existing chemotherapeutic agents. As an approach to the therapeutic attack of hypoxic cells, we have measured the cytotoxicity and DNA lesions produced by the bioreductive alkylating agents mitomycin C and porfiromycin, two structurally similar antibiotics, in oxygen-deficient and aerobic cells. Mitomycin C and porfiromycin were preferentially cytotoxic to hypoxic EMT6 cells in culture, with porfiromycin producing a greater differential kill of hypoxic EMT6 cells relative to their oxygenated counterparts than did mitomycin C. Chinese hamster ovary cells were more resistant to these quinone antibiotics; although in this cell line, porfiromycin was significantly more cytotoxic to hypoxic cells than to aerobic cells, and the degree of oxygenation did not affect the toxicity of mitomycin C.

Alkaline elution methodology was utilized to study the formation of DNA single-strand breaks and DNA interstrand cross-links produced by mitomycin C and porfiromycin in both EMT6 and Chinese hamster ovary cells. A negligible quantity of DNA single-strand breaks and DNA interstrand cross-links were produced in hypoxic and aerobic Chinese hamster ovary cells by exposure to mitomycin C or porfiromycin, a finding consistent with the considerably lower sensitivity of this cell line to these agents. In EMT6 tumor cells, no single-strand breaks appeared to be produced by these antitumor antibiotics under both hypoxic and aerobic conditions; however, a significant number of DNA interstrand cross-links were formed in this cell line following drug treatment, with substantially more DNA interstrand cross-linking being produced under hypoxic conditions. Mitomycin C and porfiromycin caused the same amount of cross-linking under conditions of oxygen deficiency; however, mitomycin C produced considerably more DNA cross-linking than did porfiromycin in oxygenated cells. DNA interstrand cross-links were observed in hypoxic EMT6 cells throughout a 24-h period following removal of mitomycin C and porfiromycin, with a decrease in DNA interstrand cross-links observed at 24 h. An increase in DNA interstrand cross-links occurred in aerobic EMT6 cells treated with mitomycin C and porfiromycin at 6 h after drug removal, with a decrease in these lesions being observed by 24 h, suggesting that the rate of formation of the cross-links may be slower and the removal of cross-links more rapid under aerobic conditions. These results were consistent with the degree of cytotoxicity produced by these agents to aerobic EMT6 cells.

INTRODUCTION

Solid neoplasms are known to contain deficient or poorly functional vascular beds and areas of severe vascular insufficiency and as a result may develop regions containing hypoxic tumor cells (1-3). These malignant cells, which frequently constitute 5 to 30% of the total viable tumor cell population (4), may form a therapeutically resistant group within solid tumors; thus, hypoxic neoplastic cells may be capable of proliferating and causing tumor regrowth after treatment that produces tumor regression. Our laboratory has suggested that hypoxic cells in solid tumors may exist in an environment conducive to reductive processes, which might be exploited by the use of chemotherapeutic agents that become cytotoxic after reductive activation (5, 6). This class of drugs, which we have called bioreductive alkylating agents, consists of compounds which require metabolic reduction to form species capable of alkylating critical cellular macromolecules. The expectation is that hypoxic tumor cells would readily activate and be preferentially susceptible to drugs of this class.

MC, an antineoplastic antibiotic in clinical use for the treatment of a variety of solid tumors (7, 8) and the related antibiotic, PM, are prototypic bioreductive alkylating agents. Previous studies by our laboratory and by others have demonstrated that a variety of cultured cell lines are more sensitive to the mitomycin antibiotics under hypoxic conditions than in the presence of air (9-17). The mitomycin antibiotics are thought to exert their cytotoxic activity by the formation of interstrand cross-links between complementary strands of DNA (18-32); these investigations have demonstrated that the highest degree of cross-linking occurs in DNAs of high guanine and cytosine content (22, 24, 27); furthermore, the cross-linking of DNA by MC is enhanced as the pH of the environment is lowered, suggesting that preferential activation of this antibiotic could occur in tumor cells which may tend to have a lower pH than normal cells (24, 33, 34). Of major significance is the evidence by several investigators (18, 20, 23, 35) that the rate of removal of interstrand cross-links by mammalian cells is important to the sensitivity of cells to MC. While the production of DNA interstrand cross-links is believed to be part of the mechanism by which MC and PM exert their cytotoxicity, no direct evidence exists that these lesions are responsible for the antineoplastic activity of these antibiotics. The present report provides more extensive data on the toxicity of MC and PM to aerobic and hypoxic EMT6 and CHO cells and, in addition, since these antibiotics are believed to exert their antineoplastic activity by the formation of DNA interstrand cross-links, utilizes alkaline elution methodology to assess the importance of these DNA lesions to the cytotoxicity of these drugs under conditions of hypoxia and normal aeration.

MATERIALS AND METHODS

Cultured Cell Lines. EMT6 mouse mammary tumor cells (subline EMT6-Rw supplied by Dr. Sara Rockwell, Department of Therapeutic Radiology, Yale University) and CHO cells (subline HA-1 supplied by Dr. Daniel S. Kapp, Department of Therapeutic Radiology, Yale University) were grown at 37°C in Waymouth's medium (Grand Island Biological Co., Grand Island, NY) supplemented with 15% fetal bovine serum (Grand Island Biological Co., Grand Island, NY) and antibiotics in a humidified atmosphere of 95% air/5% CO2. Stock cultures of EMT6 and CHO

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cells were seeded in 25-cm² plastic flasks at 5 to 10 x 10⁶ and 1 to 2 x 10⁹ cells/ml of medium, respectively, with subculturing carried out every 3 to 4 days. Other characteristics of these cell lines have been described in detail previously (36, 37).

Cell Survival Studies. To monitor cell survival, 2 x 10⁶ EMT6 cells and 3 to 4 x 10⁶ CHO cells/10 ml of Waymouth's medium supplemented with 15% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 µg/ml), were seeded in glass milk dilution bottles, and cultures were allowed to grow for 3 days, at which time the cells were in mid-exponential growth. The medium was then removed and replaced with 5 ml of fresh medium. The bottles were sealed with sterile rubber sleeve stoppers (Aldrich Chemical Co., Milwaukee, WI) and fitted with 13-gauge needles for gas inflow and 18-gauge needles for gas outflow; tubing connected to the outflow needle was immersed in water to allow visible monitoring of gas flow and to prevent back flow of air into cultures. To produce hypoxia, cultures were gassed continuously for 3 h at 37°C with a humidified mixture of 95% N₂/5% CO₂ (Presto Welding Service Centers, North Haven, CT). This incubation technique produces full radiobiological hypoxia (oxygen content, 10 ppm) after 2 h of exposure to 95% N₂/5% CO₂. Normally aerated cultures were incubated in a humidified atmosphere of 95% air/5% CO₂ (Presto Welding Service Centers). At this time, appropriate dilutions of either MC or PM (the generous gifts of Dr. T. W. Doyle, Bristol Laboratories, Syracuse, NY) dissolved in ethanol (final concentration of ethanol = 0.35%) were added directly to cultures without breaking the hypoxia, by injecting a small volume of drug through the rubber sleeve. After exposure to each drug for 1 h under hypoxia or air, cells were washed twice with 5 ml of sterile PBS, and treated with 0.05% trypsin (Grand Island Biological Co.) in PBS for 10 min. Cells were collected by centrifugation and appropriate dilutions were plated in replicate dishes and allowed to form colonies for 10 to 14 days. Colonies were stained with Gram's crystal violet (Fischer Scientific Co., Fairlawn, NJ) and counted. Both hypoxic and aerobic vehicle (0.35% ethanol) controls were included in each experiment; the surviving fractions for hypoxic cultures were calculated using the hypoxic controls.

Assay of Single-Strand Breaks and DNA Interstrand Cross-Links by Alkaline Elution. EMT6 and CHO cells were seeded in glass milk dilution bottles at a density of 1 to 2 x 10⁶ cells/10 ml of Waymouth's medium supplemented with 15% fetal bovine serum and antibiotics. After a 24-h incubation period, sample cells were labeled with [2-¹⁴C]thymidine (0.02 µCi/ml; 55 mCi/mmol; Amersham Corp., Arlington Heights, IL) and reference cells were labeled with [methyl-³H]-thymidine (0.05 µCi/ml; 2 Ci/mmol; Amersham Corp.). The radioactive medium was removed after 24 h and replaced with fresh medium until the start of experiments. This procedure enabled the labeled material to be incorporated into high molecular weight DNA. Alkaline elution technique has been described in detail (36, 37). Briefly, EMT6 or CHO sample cells prelabeled with [¹⁴C]thymidine were treated with 2 µCi MC or PM dissolved in ethanol for 1 h under hypoxia or air. At this time, the drug-containing medium was removed, the cell monolayer was washed with ice-cold Hanks' balanced salt solution containing glucose but no calcium or magnesium (Grand Island Biological Co.), and cells were either not irradiated or irradiated with 300 rads of X-irradiation (250 kV; 15 mA; 2 mm Al filter; dose rate, 153 rads/min; Siemens Stabilipan) in 5 ml of Hanks' balanced salt solution to measure single-strand breaks and DNA interstrand cross-links, respectively. Untreated EMT6 reference cells prelabeled with [³H]thymidine were also washed and irradiated with 300 rads when used in the assays to measure single-strand breaks and DNA interstrand cross-links. These reference cells provided DNA within each experiment with consistent elution kinetics which were not influenced by the elution rate of the experimental [¹⁴C]DNA. After X-irradiation, the cells were collected from the milk dilution bottles using a T-flask scraper (Belco Glass, Inc., Vineland, NJ), 5 ml of ice-cold medium was added to each bottle, and the cells were dispersed by gentle pipetting. Approximately 2.5 x 10⁶ ¹⁴C-labeled sample cells and a similar number of ³H-labeled reference cells were mixed, diluted in ice-cold PBS, and collected on a 0.8-μm pore size, 2-mm diameter, polycarbonate filter (Nuclepore Corp., Pleasanton, CA), which was placed on a 25-mm polyethylene filter holder (Swinney; Millipore Corp., Bedford, MA) connected to a 50-ml polystyrene Luer-lok syringe. These cells were then lysed on the filter with 5 ml of a lysis solution containing 2% SDS (99% purity; BDH Chemicals, Ltd., Poole, England), 0.025 M sodium EDTA (Fischer Scientific Co., Fair Lawn, NJ) and 0.1 M glycine (Bethesda Research Laboratories, Inc., Gaithersburg, MD), pH 10.0, which was allowed to flow through the filter by gravity. The lysate was deproteinized by placing 2 ml of 2% SDS, 0.025 M sodium EDTA, and 0.1 M glycine (pH 10.0) containing proteinase K (0.5 mg/ml; 20 mAnson units/ml; E. Merck, Darmstadt, West Germany) on the filters in the upper chamber of the filter holder. Following the addition of proteinase K, 40 ml of a solution containing tetrapropylammonium hydroxide (RSA Corp., Elmsford, NY), 0.02 M EDTA, free acid (Sigma Chemical Co., St. Louis, MO), and 0.1% SDS (pH 12.1) was placed in the syringe. Both solutions were pumped through the filter in the dark at a rate of 0.035 ml/min, allowing the proteinase digestion time to be approximately 2 h. Eluted fractions were collected at 3-h intervals for 15 h. When all of the fractions were collected, they were processed as follows. The eluting solution remaining in the funnel reservoir was gently poured off and discarded. The solution remaining in the pump tubing and filter holder was pumped at maximum speed into an empty scintillation vial. The filter was removed and placed in a scintillation vial containing 0.4 ml of 1 N HCl. Subsequently, the filter was heated at 60°C for 1 h to depurate the DNA. After heating the filter, it was cooled to room temperature and 2.5 ml of 0.4 N NaOH (which converts the apurinic sites to strand breaks) was added for 1 additional h. Finally, 10 ml of 0.4 N NaOH was added to the funnel to flush the filter holder and pump tubing and a 2.5-ml aliquot of this solution was assayed for its content of radioactivity. A 2-ml aliquot of lysis solution was obtained and water was added to this fraction and to the fractions containing 0.4 N NaOH to give a final volume of 6 ml. All fractions, except the fraction containing the filter, were mixed with 10 ml Aquassure (New England Nuclear, Boston, MA) with 0.7% glacial acetic acid. The fraction containing the filter was mixed with 5 ml Aquassure with 0.7% glacial acetic acid. Samples were counted in a Beckman LS 7500 scintillation counter.

DNA interstrand cross-linking indices were computed using the formula

\[ \text{Cross-link index} = \left( \frac{1 - f_0}{1 - r} \right)^n - 1 \]

where \( n \) and \( r \) were the fractions of the [³H]- and [¹⁴C]DNA remaining on the filter after approximately 10 h of elution (38, 39).

RESULTS

Cell Survival Studies. Measurements of the surviving fraction of hypoxic and aerobic vehicle-treated controls demonstrated a slight cytotoxicity with both cell lines after exposure to hypoxia; thus, the surviving fraction of the hypoxic vehicle-treated control cells was 0.472 and 0.534, whereas the aerobic vehicle-treated control survivals were 0.738 and 0.794 for EMT6 and CHO cells, respectively. The survival curves for aerobic and hypoxic cells treated with various concentrations of MC are shown in Figs. 1 and 2. The findings with EMT6 cells treated with MC agreed with those reported by our laboratory previously (9, 11, 12, 14, 15); thus, the survival curves for EMT6 hypoxic cells treated with various concentrations of MC are shown in Figs. 3 and 4. The findings indicate that PM and MC were almost equitoxic to hypoxic EMT6 cells, whereas PM was considerably less cytotoxic than MC to aerobic cells. For CHO cells, the results indicated significantly greater hypoxic cell...
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Exponentially growing cells were exposed to varying concentrations of drug for 1 h under conditions of normal aeration or hypoxia, and cytotoxicity was estimated by the ability of cells to form colonies as described in “Materials and Methods.” Data are expressed as a fraction of control survival of aerobic (○) or hypoxic (●) cells and represent mean and SE (bars) of three experiments.

Fig. 1. Survival of hypoxic and aerobic EMT6 cells after exposure to MC. Exponentially growing cells were exposed to varying concentrations of drug for 1 h under conditions of normal aeration or hypoxia, and cytotoxicity was estimated by the ability of cells to form colonies as described in “Materials and Methods.” Data are expressed as a fraction of control survival of aerobic (○) or hypoxic (●) cells and represent mean and SE (bars) of three experiments.

Fig. 2. Survival of hypoxic and aerobic CHO cells after exposure to MC. Exponentially growing cells were exposed to varying concentrations of drug for 1 h under conditions of normal aeration or hypoxia, and cytotoxicity was estimated by the ability of cells to form colonies as described in “Materials and Methods.” Data are expressed as a fraction of control survival of aerobic (○) or hypoxic (●) cells and represent mean and SE (bars) of three experiments.

Fig. 3. Survival of hypoxic and aerobic EMT6 cells after exposure to PM. Exponentially growing cells were exposed to varying concentrations of drug for 1 h under conditions of normal aeration or hypoxia, and cytotoxicity was estimated by the ability of cells to form colonies as described in “Materials and Methods.” Data are expressed as a fraction of control survival of aerobic (○) or hypoxic (●) cells and represent mean and SE (bars) of three experiments.

Fig. 4. Survival of hypoxic and aerobic CHO cells after exposure to PM. Exponentially growing cells were exposed to varying concentrations of drug for 1 h under conditions of normal aeration or hypoxia, and cytotoxicity was estimated by the ability of cells to form colonies as described in “Materials and Methods.” Data are expressed as a fraction of control survival of aerobic (○) or hypoxic (●) cells and represent mean and SE (bars) of three experiments.

Formation of Single-Strand Breaks and DNA Interstrand Cross-Links Produced by Antibiotic Treatment. Experiments using both hypoxic and aerobic EMT6 cells treated with 2 μM MC or PM for 1 h demonstrated differences dependent upon the degree of oxygenation; thus, although the alkaline elution profiles of aerobic and hypoxic EMT6 cells treated with either MC or PM for 1 h demonstrated no DNA single-strand breaks under either hypoxic or aerobic conditions, this cell line exhibited DNA interstrand cross-links, with substantially more DNA interstrand cross-links occurring under hypoxic conditions than with oxygenation (Fig. 5). Both antibiotics produced a comparable amount of cross-linking under hypoxic conditions, a finding consistent with the degree of cytotoxicity produced by these agents (Figs. 1 and 3). Aerobic EMT6 cells treated with MC demonstrated a slightly greater amount of interstrand cross-linking than those exposed to PM, which is also consistent with the degree of cytotoxicity produced by these drugs in air.

In the CHO cell line, neither MC nor PM caused detectable single-strand breaks under aerobic and hypoxic conditions (Fig. 6), a finding identical to that observed with EMT6 cells; however, contrary to the results obtained with the EMT6 carcinoma, negligible DNA interstrand cross-linking was produced by either antibiotic under hypoxic or aerobic conditions. These findings are consistent with studies which indicated that these antibiotics caused considerably less toxicity to CHO cells.

Measurement of the formation and removal of single-strand
cross-links were observed immediately following MC treatment
in hypoxic EMT6 cells; however, an increase in DNA interstrand
cross-links after MC and PM treatments, respectively, in aerobic EMT6
cells; maintained over the 24-h postincubation period used, with approximately an equivalent amount of
cross-linking occurring over the 0 to 12-h period and fewer
cross-links at 24 h (Figs. 7 and 8). No single-strand breaks
could be detected in EMT6 cells under either hypoxia or air
throughout the 0 to 24-h period after drug removal. Few detec-
table DNA interstrand cross-links were present immediately
after MC and PM treatments, respectively, in aerobic EMT6
cells; however, an increase in DNA interstrand cross-links
occurred at 6 h postincubation for both antibiotics, with the
number of cross-links decreasing during the 12- to 24-h period
following drug exposure. Considerably more DNA interstrand
cross-links were observed immediately following MC treatment
of hypoxic EMT6 cells. There were, however, no significant
differences between hypoxic and aerobic EMT6 cells in the
amount of DNA interstrand cross-links occurring 6, 12, and 24
h after drug removal. In contrast, significantly more DNA
interstrand cross-links were observed in hypoxic EMT6 than
aerobic EMT6 cells at 0, 6, and 12 h after removal of PM.

DISCUSSION

Previous work by our laboratory has demonstrated that MC
and PM are more toxic to hypoxic EMT6 cells than to their
aerobic counterparts; MC was shown to be essentially equitoxic
to hypoxic and aerobic CHO cells in culture (9, 11, 14–16). The
results of the present study extend these findings on the
cytotoxicity of MC and PM and confirm the earlier investiga-
tions which demonstrate that these agents are preferentially
cytotoxic to hypoxic and aerobic CHO cells in culture (9, 11, 14–16).
of Rauth et al. (13) who demonstrated that MC (1 µg/ml) was only slightly toxic to hypoxic and aerobic CHO cells. Measurement of the toxicity of PM to hypoxic and aerobic cultured cells yielded results different from those of MC. PM produced relatively great hypoxic cell cytotoxicity to EMT6 carcinoma cells but exhibited considerably less aerobic toxicity than that caused by MC. CHO cells treated with PM were also more susceptible under conditions of hypoxia. These findings agree with earlier studies which demonstrated that PM was an active antitumor drug but with lower potency and less toxicity to normal tissue than MC (11, 40). That the results may be related to differences in the susceptibilities of the two antibiotics to reductive activation was suggested by the findings of Patrick et al. (41) that hydrogenation of the MC related material mitomycin B over a platinum catalyst at atmospheric pressure in dimethylformamide produced an aziridinomitosene, whereas hydrogenation of PM under the same conditions gave no such product. Since both of these antibiotics have identical redox potentials, it is not clear why differences should exist between these agents in their susceptibility to reduction.

Early studies have demonstrated cross-linking of bacterial DNA in situ and cross-linking of purified bacterial DNA in the presence of cell lysates in vitro after exposure to MC (21, 26). Iyer and Szybalski (22, 27) have demonstrated that either chemical or enzymatic reduction of MC and PM was essential for cross-linking of bacterial DNA in vitro, and an increase in cross-linking occurred when bacterial DNA of high guanine-cytosine content was used. These investigators extended their findings to include studies on the production of cross-links in DNA using a cultured human cell line (26, 27). An amount of DNA cross-linking in mammalian cells in vitro and in vivo comparable to that obtained with bacterial DNA in vitro and in vivo was not obtained, even at relatively high concentrations of MC, unless purified DNA which had been deproteinized was used. [14C]PM was used to prove covalent binding of drug to bacterial DNA (28). Other investigators have confirmed the covalent binding of [14C]PM to DNA from other bacterial sources, and additionally, demonstrated covalent binding of [3H]MC to DNA (42–44). Using synthetic polynucleotides, both [14C]PM and [3H]PM were found to bind to guanine polynucleotides to a greater extent than to other polynucleotides (42, 45). The preference of MC for the guanine-cytosine base pair in DNA cross-linking was confirmed directly by Lown et al. (24) using an ethidium bromide fluorescence assay. Fujiwara (18) and Fujiwara and Tatsumi (20) observed DNA-DNA interstrand cross-links with MC-treated normal human fibroblasts using alkaline sucrose sedimentation, hydroxyapatite chromatography, and S1-nuclease digestion methodologies, while Forance and Little (46) and Forance et al. (47) observed DNA-DNA and DNA-protein cross-links with MC-treated human fibroblasts using alkaline elution methodology. In recent years, several investigators have been able to identify MC adducts after chemical or enzymatic reduction of the drug in the presence of nucleotides, calf thymus DNA, and rat liver DNA (48–51). While alkylation of DNA and the production of DNA-DNA interstrand cross-links are believed to be part of the mechanism by which MC and PM exert their anaerobic cytotoxicities, no direct evidence exists that these lesions are responsible for the antineoplastic activity of these antibiotics.

The alkaline elution technique developed by Kohn et al. (38, 39) was utilized to study the relationship of DNA single-strand breaks and DNA interstrand cross-links produced by MC and PM to the toxicity of these antibiotics to hypoxic and aerobic EMT6 and CHO cells. The results of the alkaline elution studies suggest a relationship between the cytotoxicity of these drugs and the formation of DNA interstrand cross-links. Thus, in both cell lines, single-strand breaks were not detected; however, the DNA interstrand cross-links present in antibiotic-treated EMT6 cells may have masked any single-strand breaks which may have occurred during or after drug treatments by retention of the DNA on the filter. Furthermore, even though MC is known to form hydrogen peroxide and hydroxyl radicals after reductive activation (52–54) and these radicals may cause single-strand breaks, Bradley and Erickson (55) have reported rapid repair rates for single-strand breaks induced by hydrogen peroxide in V79 cells. MC was more toxic to hypoxic EMT6 cells than to corresponding aerobic cells and more DNA interstrand cross-linking was observed under hypoxic conditions than under aerobic conditions immediately following drug treatment. A similar pattern was also observed in PM-treated EMT6 cells. Furthermore, the aerobic toxicity that occurs with MC in EMT6 cells is greater than that produced by PM, and the degree of DNA cross-linking under these conditions corresponds with these results.

Both MC and PM produced DNA interstrand cross-links in EMT6 cells that persisted over a 24-h period following drug removal. Interestingly, maximum formation of DNA interstrand cross-links in aerobic EMT6 cells occurred several hours after drug removal with both MC and PM, whereas under hypoxic conditions, maximum cross-linking of DNA was observed at the time of drug removal. With MC, the amount of interstrand cross-linking observed in aerobic EMT6 cells was comparable to that occurring in hypoxic cells at 6 and 12 h after drug removal, indicating that the rate of formation of interstrand cross-links in EMT6 cells is slower under air. Substantially fewer DNA interstrand cross-links were observed at 24 h after MC or PM removal in hypoxic and aerobic EMT6 cells. These results may indicate that removal of cross-links is taking place in these cells by repair processes, although it is possible that cell division occurred and that the decrease in the number of cross-links reflects the fact that the original population of cells was diluted at 24 h after drug exposure. Although a relationship between DNA interstrand cross-links and cytotoxicity to the mitomycin antibiotics appears to exist, other factors also must be considered; thus, it is possible that cross-linking of other cellular macromolecules, including cell membrane components, structural proteins, and enzymes may occur at highly sensitive sites in these molecules and lead to the differential cytotoxicity observed in hypoxia and normal aeration.

In conclusion, the studies presented in this report demonstrate a relationship between the cytotoxicity of the mitomycin antibiotics in hypoxic and aerobic cell lines and the degree of DNA interstrand cross-linking: thus, porfomycin is more selective for hypoxic cells than is MC, both with respect to its capacity to kill cells and to cross-link DNA. CHO cells are relatively resistant to the cytotoxic effects of MC and PM compared to EMT6 cells, and relatively few DNA interstrand cross-links were produced in CHO cells by these agents. In EMT6 cells, however, which are considerably more sensitive to the mitomycin antibiotics, particularly under hypoxic conditions, these drugs produced a much greater degree of DNA interstrand cross-linking, although the finding that the total number of DNA cross-links increases with time in oxygenated EMT6 cells to the maximum level found in hypoxic cells indicates that factors such as repair of these lesions may be of importance in the differential sensitivity of hypoxic and aerated cells to these agents. Further studies are required to clarify the
role of repair in the differential sensitivity of oxygenated and hypoxic cells to the mitomycins as well as to ascertain the reason for the relatively great cytotoxicity of MC and PM towards hypoxic EMT6 cells compared to the relative resistance of CHO cells. It is conceivable that differences in factors such as uptake, activation, and/or subsequent reaction with DNA are responsible for the different sensitivities of the two cell lines to these agents.

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REFERENCES

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