Role of Hypoxia in Anticancer Drug-induced Cytotoxicity for Ehrlich Ascites Cells

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ABSTRACT

A review of the literature on the effect of hypoxia on in vitro drug sensitivity had suggested that there was consistently more cytotoxicity under hypoxic conditions for the drugs misonidazole and mitomycin C while there was much conflicting data for the drugs Adriamycin and bleomycin. We have examined the effect of oxygen on the cellular response of Ehrlich's ascites tumor cells to the drugs mitomycin C, misonidazole, Adriamycin, and bleomycin. Significant differences were observed when we compared the cytotoxicity of mitomycin C and misonidazole as a function of oxygen concentration. For the drugs Adriamycin and bleomycin no differential effects of oxygen were noted for a 1-h drug exposure with hypoxia while some differences were noted only for bleomycin for an 8-h drug exposure time. Because differences dependent on oxygen concentration were observed for some drugs but not others at the same experimental conditions, and as indicated by a review of the literature, it is suggested that some of the conflicting data in the literature with respect to some of these drugs may be cell-line dependent. Other variables which may also be of importance were the duration of drug exposure time in hypoxia and cell density. The observed oxygen concentration-dependent changes in cell survival for the Ehrlich cells with drugs examined could not be explained on the basis of changes in drug-induced cellular oxygen consumption.

INTRODUCTION

Oxygen microenvironment in tumors is heterogeneous (1, 2). It is known that cell killing by radiation is dependent upon oxygen concentration and that hypoxic cells are resistant to radiation (3). Hypoxic cells may also be resistant to drugs because of drug delivery problems secondary to inadequate vasculature (4) and/or intrinsic effects of hypoxia on cell response to drugs (5). There is some evidence that oxygen concentration may play a significant role in drug-induced cell killing (5). Drugs such as mitomycin C and misonidazole are known to be selectively metabolized under hypoxic conditions to active compounds (6, 7) while drugs such as Adriamycin and bleomycin may be cytotoxic through oxygen radical-mediated mechanisms (8, 9). Pre-, during, and postdrug treatment influences of varying oxygen concentrations have all been described in the literature (10, 11). The effect of oxygen concentration on the cytotoxicity of misonidazole has been established (12, 13) and similar studies with mitomycin C have been recently published (14). However, the in vitro data are conflicting with the exception of misonidazole. Because of the conflicting data summarized in Table 1 we have examined the effect of oxygen concentration on cell survival for the anticancer agents mitomycin C, misonidazole, Adriamycin, and bleomycin.

MATERIALS AND METHODS

Tumor line used for the experiments was a tetraploid Ehrlich's ascites tumor (Mason Research Institute, Worcester, MA) which was main

tained by i.p. injection of 1 x 10⁷ cells weekly in ICR white mice. Cells from animals bearing 6–8-day old ascites tumors were used. In vitro studies on the effect of oxygen on drug-induced cell survival were performed as follows. EAT² cells (1–5 x 10⁶) were suspended in 10 ml of Hams F10 media in 25-ml Erlenmeyer glass flasks. The flasks were sealed with rubber sleeve stoppers and fitted with two 21-gauge needles for an inlet and outlet port. The flasks were gassed with oxygen mixture of interest at flow rates of 1 liter/min for 1.5 h at 37°C on an Orbital Shaker, model 3520 (Lab-Line Instruments, Inc.) at 1800 rpm. This is similar to the method reported by Mohindra and Rauth (15). Gas mixtures used contained 0–20% O₂, 5% CO₂, and balance N₂ (Linde Corp., Houston, TX). Following this preequilibration of oxygen, drugs were added directly without opening the flask by using a 21-gauge needle in a 0.1-ml volume. The effect of both short and prolonged drug exposure time during hypoxia on drug cytotoxicity was examined in some cases. For the 1-h drug exposure a cell density of 1 x 10⁶/ml was used without humidification. Measurement of volume of media failed to reveal any significant effect of evaporation. For the 8-h drug exposure under hypoxia, a cell density of 5 x 10⁶/ml was used with humidification as this time period would have resulted in significant evaporation. In some instances cells were exposed to a humidified preincubation atmosphere of 5% O₂ (oxic) or N₂ (hypoxic) for 16.5 h before drug incubation for 1 h at the stated oxygen atmosphere in order to determine whether the duration of hypoxia before drug exposure influences cytotoxicity. After drug incubation with continuous gassing, the cells were centrifuged, washed with 10 ml of media, and resuspended in fresh Hams F10 media for plating. The colony assay for the EAT cells has been described previously (32). Tumor cells were suspended in Hams F10 media containing 15% fetal calf serum, 1% penicillin-streptomycin, and 0.3% agar and plated in 35-mm Petri dishes in a 2-ml volume. The dishes were then incubated in humidified Modulator Incubator Chambers (Billups-Rothenberg Inc., Del Mar, CA) and flushed with 5% O₂, 5% CO₂, and 90% N₂. A 5% O₂ atmosphere was used for the plating because we had previously found there was no growth of the EAT cells at 20% O₂ in the absence of antioxidants (32). Seven to 8 days later, plates were scored for colony formation (>50 cells).

Oxygen measurements in solution were performed using a Diamond-Electrotech, Inc. (Ann Arbor, MI) oxygen measuring system which consisted of an amplifier unit (no. 1201), calibration cell (no. 1251), and Clarke type oxygen electrode. Oxygen measurements were made in separate experiments with and without cells similar to the drug experiments except that phosphate buffered saline was used. For each experiment a separate calibration curve using N₂ and 0.1, 1, 5, 10, and 21% O₂ was performed. The current was recorded as a function of time and the oxygen concentration calculated from the calibration curve. Using this system, oxygen level below 0.1% cannot be measured accurately. The data was plotted according to an equation (Equation A) described by Whillans and Rauth (33):

\[
(C_i - C_f) = (C_i - C_0)e^{-kt}
\]

where \(C_i\) is the oxygen tension in solution at time \(t\), \(C_f\) is the initial oxygen tension in solution, \(C_0\) is the oxygen concentration at equilibrium, and \(k_t\) is a physical constant independent of oxygen concentration and depletion rate. From the measured values of oxygen consumption rate \(R\) and \(k_t\), one can calculate the effect of oxygen depletion on the oxygen concentration at equilibrium using the relationship \(C_e = C_i - R/k_t\) (33) where \(C_e\) represents oxygen concentration in the gas phase.

Oxygen consumption studies were performed on a Gilson Oxygraph located in the Division of Radiation Research, University of Texas
### HYPOXIA AND DRUG CYTOTOXICITY

#### Table 1 Summary of literature on the effect of oxygen concentration on drug cytotoxicity

<table>
<thead>
<tr>
<th>Drug</th>
<th>Preferentially toxic to oxic cells</th>
<th>Preferentially toxic to hypoxic cells</th>
<th>No difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Misonidazole</td>
<td>CHO* (12) EMT6 (13)</td>
<td>EMT6 (10, 16–19), CHO (14, 20), V79, KHT, HeLa (20), S180 (17), EAT* and P388*</td>
<td>CHO (16)</td>
</tr>
<tr>
<td>Metronidazole</td>
<td></td>
<td></td>
<td>EMT6 (21)</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>EMT6 (10)</td>
<td></td>
<td>EMT6 (21)</td>
</tr>
<tr>
<td>Procarbazine</td>
<td>EMT6 (10)</td>
<td></td>
<td>EMT6 (21)</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>EMT6 (10)</td>
<td></td>
<td>EMT6 (21)</td>
</tr>
<tr>
<td>Vincristine</td>
<td>EMT6 (17)</td>
<td></td>
<td>EMT6 (24)</td>
</tr>
<tr>
<td>Streptonigrin</td>
<td>EMT6 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleomycin</td>
<td>V79 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adriamycin</td>
<td>B14 FAF28 (25)</td>
<td>EMT6 (10, 17)</td>
<td>EMT6 (21)</td>
</tr>
<tr>
<td>Bisantrene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTIC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>CHO (29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCNU</td>
<td>V79 (30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>EMT6 (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Methotrexate</td>
<td></td>
<td></td>
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<tr>
<td>Etoposide</td>
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<td></td>
<td></td>
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<tr>
<td>Alkeran</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nitrogen mustard</td>
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</tbody>
</table>

* CHO, Chinese hamster ovary; DTIC, 5-(3,3-dimethyl-1-trieno)imidazole-4-carboxamide; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; EMT6, mouse mammary tumor cells; V79, B14 FAF28, Chinese hamster; S180, KHT, mouse sarcoma cells; P388 and L1210, mouse leukemic cells; HeLa, WiDR, HEC-1A, human epithelial cancer cell lines; HTC, human tumor biopsy cells.

* This study.

Medical Branch, Galveston, TX, through the courtesy of Dr. Neil Howell. Oxygen consumption rates at 21% O₂ were determined with and without the drugs Adriamycin, bleomycin, and mitomycin C at 50 µg/ml directly inside the oxygen consumption chamber. In addition, mitomycin C (3 µg/ml)- and misonidazole (1000 µg/ml)-treated cells at 0.01% O₂ for 1 h were washed free of drug, resuspended in media, and the O₂ consumption rates were determined.

### RESULTS

Measurements of oxygen tension in solution without cells with time were used to determine the physical constant k₁ according to data in Fig. 1. A k₁ value of 0.55 min⁻¹ with a half-time of 1.26 min for equilibration was obtained. The former value is considerably higher than previously reported systems (33, 34) and reflects the high degree of gas exchange occurring in our system. This can be explained on the basis of geometry of vessel used (33, 34), and the high rate of shaking causing sufficient dispersion of the fluid over the glass surface and thereby decreasing the depth of the fluid.

The respiratory rate of the EAT cells at 37°C was 1.312 ± 0.179 nmol O₂/min/10⁶ cells. This is similar to the previously quoted respiratory rate of about 0.1% O₂/min/10⁶ cells for these cells (34, 35).

From the experimental values of k₁ and R, the theoretical oxygen level in solution at equilibrium (Cₘ) was calculated for a cell density of 1 x 10⁵/ml. Calculated Cₘ values for Cₘ of 20, 10, 5, 1, 0.1, and 0.01% O₂ were 19.985, 9.985, 4.985, 4.985, 0.985, 0.085, and 0% O₂, respectively. The latter value may not be correct as the oxygen consumption rate changes in the 0.01–0.1% O₂ range (12, 35, 36). At 0.01% O₂ the oxygen consumption rate for EAT cells decreases to about 25% of initial value (35). This would result in a Cₘ value of 0.0064% O₂ for a 0.01% O₂ atmosphere. At the cell density of 1 x 10⁵/ml used experimentally in Figs. 2 and 4, we were unable to detect any significant differences in the measured oxygen levels with or without cells for a 1% O₂ atmosphere. Similar measurements at 0.1% O₂ were not attempted because this represents the limit of our oxygen measuring system.

The effect of oxygen concentration on the cytotoxicity of mitomycin C for the EAT cells is shown in Fig. 2. There was an increase in the cytotoxicity of mitomycin C as the oxygen concentration was decreased to 0.01% O₂. At the higher drug concentrations of mitomycin C (2 and 3 µg/ml) there was no apparent difference in the cytotoxicity at 0.1% or 0.01% O₂.
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Fig. 2. Surviving fraction of $1 \times 10^6$/ml EAT cells treated with mitomycin C for 1 h after preincubation with different oxygen concentrations for 1.5 h. Bars, mean ± SE of three to five experiments.

Fig. 3. Relative drug concentration of mitomycin C necessary as a function of oxygen concentration to achieve equivalent cell killing. Data extrapolated from Fig. 2. The data are expressed as a ratio of drug concentration necessary to reduce cell survival to 0.31($D_0$) for the various oxygen levels when compared to 0.01% O$_2$.

There was also no difference in survival curves between 0.01% O$_2$ or N$_2$ atmosphere (data not shown).

The relative increase in drug concentration of mitomycin C necessary to achieve equivalent reduction of cell survival is plotted in Fig. 3 based on the data in Fig. 2. The half-maximal increase in drug concentration to achieve equivalent cell killing occurs at 4.0% O$_2$. This is different than what is observed with radiation-induced cell killing where cell survival decreases as the oxygen concentration is increased with a half-maximal value of approximately 0.5% O$_2$.

These data with mitomycin C are in contrast to the influence of oxygen concentration on the cytotoxic effects of misonidazole in Fig. 4. There is an apparent stringent requirement for extreme hypoxia for the induction of the cytotoxicity of misonidazole. Differences between survival curves of misonidazole and mitomycin C are apparent for N$_2$ and 0.01 and 0.1% O$_2$ atmosphere (Figs. 2 and 4). Similar experiments with Adriamycin and bleomycin (Figs. 5 and 6) failed to reveal significant differences in the cytotoxicity between the N$_2$ and 20% O$_2$ atmosphere for a 1-h drug exposure. However, significant differences in cell survival were observed for a more prolonged drug exposure (8 h) during hypoxia for bleomycin (Fig. 7) but not Adriamycin (Fig. 8) under similar experimental conditions. EAT cells exposed for 16.5 h to a 5% O$_2$ or N$_2$ atmosphere before treatment with 1 $\mu$g/ml of Adriamycin for 1 h under hypoxic andoxic conditions failed to reveal significant differences in cell survival (Table 2). The cell survival values obtained are comparable to that shown in Fig. 5 for the same drug concentration but for a much shorter duration of preincubation hypoxia. For cells exposed to a 5% O$_2$ or N$_2$ atmosphere for 16.5 h (Table 2) differences in the plating efficiency of drug-unprotected cells, in cell cycle distribution by flow cytometry, or in uptake of tritiated thymidine were not observed (data not shown).

The effects of cell density and pH on bleomycin cytotoxicity were determined because these variables may affect the cytotoxicity of bleomycin if different cell densities are used under hypoxic andoxic conditions. As can be seen from Table 3, there were significant differences with the relative survival fraction increasing with cell density. We were unable to demonstrate a significant effect of pH on bleomycin cytotoxicity over a range of 6.49–7.22 (data not shown).

In order to determine whether some of the differences in the cell survival curves as a function of oxygen concentration with the drugs mitomycin C and misonidazole were due to changes in drug-induced cell oxygen consumption rates, measurements of oxygen consumption were performed under conditions similar to that utilized in the experiments. We were unable to demonstrate any significant effects. In addition, we were also unable to demonstrate significant changes in cellular oxygen consumption after the addition of extremely high drug concen-
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Fig. 6. Same as in Fig. 5 except bleomycin was used. Points, mean ± SE of three experiments.

Fig. 7. Surviving fraction of 5 × 10^6/ml EAT cells treated with 10 μg/ml bleomycin for 2-8 h drug exposure time after initial 1.5 h preincubation time at 20% O_2 or N_2. Points, mean ± SE of four experiments. pH measurements under oxic and hypoxic conditions for the 8-h time period varied from 7.13 to 7.22 and 7.13 to 7.09, respectively. Differences at 8-h point only were significant (P < 0.053) by analysis of variance.

trations (50 μg/ml) of Adriamycin, bleomycin, and mitomycin C. These O_2 cell consumption rates were 12.78 ± 2.14, 13.00 ± 2.12, and 14.30 ± 2.65 nmol O_2/min/10^7 cells, respectively, when compared to a control respiratory rate of 13.12 ± 1.79 nmol O_2/min/10^7 cells.

DISCUSSION

The drug misonidazole was first studied because there are consistent data in the literature demonstrating its selective toxicity towards hypoxic cells in vitro. In addition, the oxygen concentration-dependent cytotoxicity of misonidazole had been established and had indicated that it is most toxic at oxygen concentrations less than 0.1% O_2 (12, 13). The data presented in Fig. 4 confirm these observations and indicate that the experimental conditions used in the present study were sufficient to demonstrate biological differences in the oxygen concentration-dependent cytotoxicity of anticancer agents.

The data in Figs. 2 and 3 clearly demonstrate that the cytotoxicity of mitomycin C for the EAT cells is highly oxygen concentration dependent, being more toxic to hypoxic than well-oxygenated cells. For the EAT cells there was very little difference in the cytotoxicity of mitomycin C in the 0.0-0.1% O_2 range. These data are in contrast to recently published data by Marshall and Rauth (14). These investigators observed that most of the change in toxicity of mitomycin C under hypoxic conditions occurred in the 0.001-1% O_2 range for Chinese hamster ovary cells. Moreover, these differences were apparent after a 5-h drug exposure with very little difference observed at 1 h, in contrast to our data with a 1-h drug exposure time. An examination of cell survival curves from other studies would also suggest that quantitative differences in the effect of mitomycin C under hypoxic and oxic conditions are in some instances a function of duration of drug exposure time (16, 20).

In this regard it has previously been shown that the duration of hypoxia before drug exposure is not an important variable for mitomycin C cytotoxicity (37). Some work published by Ludwig

Table 2 Effect of prolonged hypoxia on Adriamycin cytotoxicity

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Oxygen concentration</th>
<th>pH</th>
<th>Surviving fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5% O_2</td>
<td>6.83</td>
<td>0.010</td>
</tr>
<tr>
<td>2*</td>
<td>N_2</td>
<td>6.49</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>5% O_2</td>
<td>6.69</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>N_2</td>
<td>6.50</td>
<td>0.036</td>
</tr>
</tbody>
</table>

* No changes in cell cycle distribution by flow cytometry or uptake of tritiated thymidine at end of experiment for control cells.

Table 3 Effect of cell density on bleomycin cytotoxicity

<table>
<thead>
<tr>
<th>Cell density</th>
<th>Relative increase in survival fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 × 10^4</td>
<td>1.21 ± 0.40*</td>
</tr>
<tr>
<td>1 × 10^5</td>
<td>1.68 ± 0.08</td>
</tr>
<tr>
<td>1 × 10^6</td>
<td>4.35 ± 2.00</td>
</tr>
</tbody>
</table>

* Mean ± SE of three to five experiments.
et al. (19) would suggest that the enhanced cytotoxicity of mitomycin C under hypoxic conditions may be cell line dependent. These workers were unable to demonstrate a significant effect of mitomycin C under hypoxic conditions at clinically achievable drug concentrations for human tumor biopsy specimens, WiDR and HEC-1A human tumor cell lines, while demonstrating a significant effect for EMT6 and S180 murine tumor cells. These data, if confirmed, may have significant clinical implications for the use of mitomycin C to selectively kill hypoxic tumor cells.

The increased metabolism of mitomycin C under hypoxic conditions to alkylating intermediates is well known and forms the basis for the increased cytotoxicity under hypoxic conditions (5, 6). Although one can demonstrate that rate of formation of alkylating intermediates is higher under hypoxic than aerobic conditions, there is no quantitative correlation between these intermediates and the extent of cell killing under hypoxic conditions (16). These data would suggest that factors other than, or in addition to, the formation of alkylating intermediates may influence cytotoxicity of mitomycin C under hypoxic conditions. Such reasons may explain the differences in the oxygen concentration dependency curves of mitomycin C between our data and that of Marshall and Rauth (14). In fact, Ludwig et al. (19) in their study were unable to demonstrate the enhanced disappearance of mitomycin C under hypoxic conditions for the human cell line WiDR suggesting that the enzymes responsible for enhanced metabolism under hypoxic conditions may not be present in some human tumor cell lines.

Because hypoxic cells are radiation resistant, the use of mitomycin C as an adjunct to selectively kill hypoxic cells has been proposed for clinical use (5). However, animal studies have failed to demonstrate any significant selective cytotoxicity of mitomycin C for hypoxic cells in vivo (20, 38, 39). The oxygen concentrations in normal and tumor tissues are heterogeneous and reported to be in the range of 2–5% O2 and 0.1–5% O2, respectively (1, 2, 40). Estimates of the hypoxic fraction in tumors range from 0–100% (41). These observations and our data indicating significant cytotoxicity of mitomycin C atoxic and intermediate levels of hypoxia may help to explain the difficulty in trying to demonstrate a selective effect of mitomycin C on hypoxic cells in vivo.

Our data raise the question why there are differences in the oxygen concentration dependency between misonidazole and mitomycin C. Misonidazole is metabolized under hypoxic conditions by a nitroreductase (5). The nitroreduction is highly sensitive to inhibition by rather low concentrations of oxygen and the extent of inhibition appears to be drug dependent (42). The metabolism of mitomycin C to reactive alkylating intermediates is enhanced under hypoxic conditions (14, 16). These differences in drug metabolism between mitomycin C and misonidazole may help to explain the differences in the oxygen concentration-dependent cytotoxicities observed with these drugs. Differences in the oxygen concentration dependency between mitomycin C and misonidazole may be explained on the basis of changes in drug-induced oxygen consumption since we did not observe significant differences in these measurements.

The duration of hypoxia and drug exposure time (25, 26), cell density (25, 43), pH (25, 30), and proliferative status (44) are some variables which may influence the response of cells to drugs under hypoxic conditions. The conflicting data published in the literature (Table 1) would suggest that the cytotoxicity of bleomycin is enhanced under oxygenated conditions for 1-h (10), 4-h (22), and 4–8-h (23) drug exposure time. There are two studies where no differences were observed for a 1–2-h drug exposure time (23, 24). Our data clearly indicate that for the EAT cells the drug exposure time in hypoxia was important for the expression of cytotoxic effects of bleomycin. Such cells were more resistant than oxygenated cells with time. We found that the EAT cells were also more resistant to bleomycin as the cell density increased. This is similar to the effects of cell density on Adriamycin cytotoxicity (25, 43). Since some investigators have used different cell densities underoxic and hypoxic conditions, it is possible that this may explain some of the quantitative differences in the literature. Our data and that of the literature does not explain why hypoxic cells should be more resistant to bleomycin than oxygenated cells with time. The observed differences are difficult to explain on the basis of a cellular oxygen radical-mediated mechanism. This is supported by the fact that differences in the cytotoxic effects of bleomycin for a 1-h drug exposure time or significant changes in cellular oxygen consumption rates at rather high drug concentrations of bleomycin were not observed. The cell survival differences with bleomycin after 8-h exposure to hypoxia cannot also be explained on the basis of changes in proliferative characteristics since EAT cells exposed to 16.5 h of hypoxia (Table 2) did not display significant changes in cell cycle distribution or uptake of tritiated thymidine.

The data in the literature with respect to the effect of Adriamycin under hypoxic or oxic conditions is most confusing. Some data would suggest that chronically (23, 25, 26) but not acutely hypoxic cells (21, 23, 25, 26) are more resistant to Adriamycin. However, other data would indicate that Adriamycin is more cytotoxic under oxic conditions (10) or that there are no significant differences in the effect of Adriamycin between acutely and chronically hypoxic cells (21). The reason for these differences in results is not clear.

In contrast to our data with bleomycin, we were unable to demonstrate any significant difference in the cytotoxicity of Adriamycin between oxic and hypoxic conditions for 1–8 h of drug exposure time or for cells previously exposed to a 16.5-h preincubation time in hypoxia (chronic hypoxia). It is possible that some of the differences may be related to the induction of oxygen-regulated proteins which may be of importance in modulating the response to Adriamycin under hypoxic conditions (44, 45). In this regard it is interesting that prolonged exposure to metabolic inhibitors such as 2-deoxyglucose and 2,4-dinitrophenol which results in increased cell survival to Adriamycin (46) may also be associated with induction of new cell protein synthesis (47–49). Because the EAT cells are already growing in vivo under conditions of radiobiological hypoxia (50), it is possible that these cells are used for in vitro drug responses that the relative resistance to Adriamycin reported following a period of prolonged hypoxia may not be observed. In an assessment of in vivo response of hypoxic tumor cells to Adriamycin, Tannock (51) concluded that hypoxic cells were spared by Adriamycin but that this was due to drug delivery problems.

We had previously demonstrated an effect of superoxide dismutase on overcoming the effect of excess oxygen toxicity on the growth of EAT cells (32). Therefore, the effect of oxygen radical scavengers on Adriamycin-induced cell cytotoxicity was studied. We were unable to demonstrate any significant effects on Adriamycin cytotoxicity with the oxygen radical scavengers superoxide dismutase, catalase, dimethylsulfoxide, and vitamin E. Brox et al. have reported that they did not observe significant differences in DNA strand breakage due to Adriamycin under hypoxic or oxic conditions (52). We have presented data indicating that differences in Adriamycin-induced cytotoxicity under hypoxic conditions or in cell oxygen consumption rates were not present for EAT cells. These observations suggest that oxygen radical-mediated mechanisms may not be important for

V. Gupta, unpublished observations.
some tumor cells for clinically achievable drug concentrations of Adriamycin at the cell level.

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