Role of hydrogen peroxide and hydroxyl radical formation in the killing of Ehrlich tumor cells by anticancer quinones

(doxorubicin/mitomycin C/diaziridinylbenzoquinone/Ehrlich carcinoma cells)

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ABSTRACT The cytotoxicity of the clinically important antineoplastic quinones doxorubicin, mitomycin C, and diaziridinylbenzoquinone for the Ehrlich ascites carcinoma was significantly reduced or abolished by the antioxidant enzymes catalase and superoxide dismutase, the hydroxy radical scavengers dimethyl sulfoxide, diethyldithiocarbamate, and thiourea, and the iron chelators deferoxamine, 2,2'-bipyridine, and diethyl-ethylenediaminetetraacetic acid. However, tumor cell killing by 5-imidooaurubicin, a doxorubicin analog with a modified quinone function that prohibits oxidation-reduction cycling, was not ameliorated by any of the free radical scavengers tested. Furthermore, treatment of intact tumor cells with doxorubicin, mitomycin C, and diaziridinylbenzoquinone but not 5-imidooaurubicin generated the hydroxy radical, or a related chemical oxidant, in vitro in a process that required hydrogen peroxide, iron, and intact tumor cells. These results suggest that drug-induced hydrogen peroxide and hydroxyl radical production may play a role in the antineoplastic action of redox active anticancer quinones.

Quinone-containing antineoplastic agents, including doxorubicin (DOX), mitomycin C (MMC), and diaziridinylbenzoquinone (AZQ), constitute one of the most important groups of chemicals used in cancer chemotherapy because they possess significant therapeutic action in the treatment of most hematologic malignancies as well as carcinomas of the lung, breast, ovary, brain, and gastrointestinal tract (1–3). Although the mechanism of tumor cell killing by these drugs has been related to their effects on DNA structure or synthesis as a consequence of intercalation or alkylation (2–4), recent evidence suggests that cytotoxicity, at least for DOX, may also be due to lethal events occurring at extranuclear sites (5, 6). Furthermore, despite studies demonstrating reactive oxygen production and DNA damage after cyclical reduction and oxidation of the quinone function in the presence of purified NADPH-cytochrome P-450 reductase (7), NADH dehydrogenase (8), and xanthine oxidase (9, 10), conclusive evidence regarding a role of the quinone moiety in the anticancer action of these compounds for intact cells is lacking.

Quite recently, however, production of potent oxidizing species, including the hydroxy radical (·OH), has been demonstrated during treatment of intact human MCF-7 breast cancer cells with DOX (11). Furthermore, resistance to the cytotoxic effect of DOX in this cell line apparently involves, at least in part, a significant enhancement of intracellular antioxidant defense enzymes (12). These preliminary results, taken together with studies from this laboratory indicating that DOX, MMC, and AZQ stimulate superoxide anion and H₂O₂ production by microsomal, mitochondrial, and nuclear preparations from Ehrlich tumor cells, strongly suggest that oxygen radical metabolism might play a role in the antineoplastic action of the anticancer quinones (13–15).

For these reasons, the effect of a wide range of reactive oxygen scavengers on the toxicity of the major antineoplastic quinones for Ehrlich carcinoma cells was examined. Agents that detoxify H₂O₂ or ·OH, including proteins that do not penetrate the cell surface, significantly reduced or abolished the cytotoxicity of these drugs. Thus, both extracellular and intracellular oxygen radical attack could be involved in the tumor cell toxicity produced by anticancer quinones. In addition, tumor cell killing was diminished by antioxidants only for drugs that generated reactive oxygen species from intact cells, suggesting that cytotoxicity from H₂O₂ or ·OH is a specific explanation for at least part of the antineoplastic effect of these chemotherapeutic agents. The data for this study have been published in abstract form (46, 47).

MATERIALS AND METHODS

Materials. Doxorubicin hydrochloride was purchased from Adria Laboratories (Wilmington, DE). 5-Imidooaurubicin and AZQ were supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. MMC was obtained from Bristol Laboratories (Syracuse, NY). Dimethyl sulfoxide (Me₂SO), diethylenetriaminopentaacetic acid (DTPA), and superoxide dismutase (SOD) [2750 units/mg, as assayed by the method of McCord and Fridovich (16)] were purchased from Sigma. Diethyldithiocarbamate, thiourea, and urea were obtained from Aldrich. 2,2'-Bipyrididine was purchased from Baker. Dubecco's modified Eagle's medium (DME medium), Ham's nutrient mixture (F12) (F12 medium), and heat-inactivated fetal calf serum (FCS) were from Grand Island Biological (Grand Island, NY). The synthetic, selenoorganic compound 2-phenyl-1,2-benziselenazol-3(2H)-one (PZ 51), which possesses glutathione peroxidase-like activity (17, 18), was a gift of E. Graf (A. Nattermann and Cie Gmb. H, Cologne, F.R.G.). The mean iron concentration of a 1:1 (vol/vol) mixture of DME medium/F12 medium containing 10% FCS was 4 μM when four different lots of each medium were combined and examined by atomic absorption spectroscopy.

Cell Lines. Ehrlich–LeiГёttre tumor cells were maintained by weekly passage of one million cells i.p. in 20-g female Swiss–Webster mice. For experiments examining the effect of anticancer quinones on cellular ·OH production, tumor cells were harvested 5–6 days after implantation, washed twice in 0.9% NaCl, exposed to hypotonic shock lysis to remove contaminating erythrocytes (19), and resuspended in

Abbreviations: Me₂SO, dimethyl sulfoxide; DOX, doxorubicin; MMC, mitomycin C; AZQ, diaziridinylbenzoquinone; SOD, superoxide dismutase; FCS, fetal calf serum; DTPA, diethylenetriaminopentaacetic acid; PZ 51, 2-phenyl-1,2-benziselenazol-3(2H)-one; IC₅₀, drug concentration producing a 50% inhibition of clonogenic cell growth.
a 1:1 (vol/vol) mixture of DME medium and F12 medium containing 10% FCS (DME medium/F12 medium/FCS). Cell viability (routinely >95%) was confirmed by exclusion of 0.1% trypan blue dye.

For experiments examining the cytotoxic effect of anticancer quinones, Ehrlich cells were transferred to tissue culture by plating 0.5 × 10⁶ cells in 25 ml of DME medium/F12 medium/FCS supplemented with 15 mM Hepes buffer and 1.2 g of sodium bicarbonate per liter at pH 7.4; the cells were maintained in suspension culture at 37°C in a humidified 5% CO₂ in air atmosphere. Tumor cells were used for drug experiments 3 days after plating 0.5 × 10⁶ cells per 25 ml of medium in 75-cm² plastic flasks.

The catalase level of Ehrlich carcinoma cells in logarithmic-phase growth incubated for 1 hr with 3000 units of the enzyme per ml was also determined in triplicate. In these experiments, 1 × 10⁶ tumor cells were homogenized with 20 strokes of a Dounce homogenizer and centrifuged at 104,000 × g and 4°C for 60 min; the supernatant was assayed for catalase activity as described (20) and was compared, in triplicate, to paired samples incubated without exogenous catalase.

Drug Treatment and Clonogenic Cell Survival Determinations. The antitumor effect of anticancer quinones was examined by exposure of Ehrlich cells in the tissue culture flask to a constant (36 μl) volume of drug for 1 hr; treatment of the cells with either the anticancer agent or a free radical scavenging agent or both always occurred in a total volume of 35 ml of DME medium/F12 medium/FCS. Briefly, 3 days after plating, paired groups of Ehrlich tumor cells in suspension culture (1–2 × 10⁶ cells per ml) exponentially growing in DME medium/F12 medium/FCS were treated with either DME medium/F12 medium/FCS (10 ml) or an equal volume of medium with the scavenger under investigation for 1 hr; cells were then exposed to either 36 μl of medium or to an equal volume of the anticancer quinone for 1 hr. At the completion of drug exposure, cells were washed twice in fresh medium and resuspended in 5 ml of DME medium/F12 medium/FCS. The Ehrlich cells were counted in a hemocytometer and cell viability (in all cases >95%) was measured by trypan blue dye exclusion.

Cell survival after drug treatment for the tumor cells was then determined by soft agar cloning as described (21). Dilutions of 1000 and 10,000 cells were prepared in the resuspension medium and plated in triplicate in 35 × 10 and 60 × 15 mm Petri dishes, respectively. For these studies, the feeder layer consisted of DME medium/F12 medium/FCS and 0.5% agar; the overlayer contained the cells, DME medium/F12 medium/FCS, and 0.3% agarose. Colonies of >50 cells were counted 6 days after plating. The percentage of clonogenic cell survival has been expressed as the number of colonies produced by drug-treated cells divided by the number of colonies produced by control cells (corrected for cloning efficiency) × 100. The plating or cloning efficiency of the Ehrlich cells in this system [which is defined as the ratio of the number of colonies counted divided by the number of cells initially plated (22)] ranged from 40% to 75%. Detailed control experiments revealed that in the concentrations used for these studies none of the free radical scavenging agents produced any significant effect on the plating efficiency of the tumor cells.

Measurement of ·OH Production. The formation of ·OH, or an oxidizing species with the chemical reactivity of ·OH, by Ehrlich tumor cells treated with anticancer quinones was measured by measurement of CH₂ production from Me₃SO by using a modification of the method of Repine et al. (23, 24) as published recently (8). For these experiments, the 2-ml mixtures contained 2 × 10⁴ tumor cells and 100 mM Me₃SO in DME medium/F12 medium/FCS, and the concentrations of reactive oxygen scavengers and anticancer quinones shown in Table 3; reactions were initiated by addition of the anticancer quinone into the gas-tight, siliconized reaction vessels by injection through the red rubber stopper. Incubation at 37°C was for 2 hr in a shaking water bath and reactions were terminated by placing the experimental tubes on melted ice. A 500-μl sample of the headspace gas from each tube (total volume, 1 ml) was assayed for methane concentration.

Statistical Analyses. Data were analyzed with the two-tailed Student's t test for independent means [not significant, P > 0.05 (25)]. Data have been expressed as the mean ± SEM.

RESULTS

Effect of Radical Scavengers and Antioxidant Enzymes on Anticancer Quinone Cytotoxicity. The initial experiments investigated whether several potent ·OH scavengers altered the cytotoxicity of various antineoplastic quinones (Table 1). Diethylurea, thiourea, and Me₃SO significantly inhibited tumor cell killing by MMC, AZQ, and DOX; although thiourea was the most potent inhibitor of cytotoxicity, Me₃SO produced complete protection against cell killing by DOX at a concentration of 300 mM, and diethylurea produced almost total protection for cells treated with DOX or MMC. It is important to point out that Me₃SO, itself, may produce many biophysical changes in tumor cells in addition to scavenging ·OH and that these effects might make cells

<table>
<thead>
<tr>
<th>Anticancer quinine</th>
<th>Conc., μM</th>
<th>None</th>
<th>3000 U/ml</th>
<th>3000 U/ml</th>
<th>50 μg/ml</th>
<th>50 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMC</td>
<td>2.5</td>
<td>50 ± 3</td>
<td>88 ± 5</td>
<td>58 ± 5</td>
<td>89 ± 4</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>AZQ</td>
<td>7.5</td>
<td>46 ± 3</td>
<td>72 ± 5</td>
<td>49 ± 1</td>
<td>51 ± 1</td>
<td>48 ± 2</td>
</tr>
<tr>
<td>DOX</td>
<td>1.5</td>
<td>51 ± 2</td>
<td>75 ± 1</td>
<td>52 ± 2</td>
<td>72 ± 2</td>
<td>58 ± 4</td>
</tr>
</tbody>
</table>

All anticancer quinones except AZQ were initially dissolved fresh in glass-distilled, deionized water and protected from light before use. AZQ was reconstituted in a 20% (vol/vol) dimethylacetamide stock solution; when used for these experiments, the final concentration of dimethylacetamide was never above 0.02% (vol/vol), which had no effect on tumor cloning efficiency. All reactive oxygen scavengers were prepared in tissue culture medium except PZ 51, which was initially dissolved in concentrated form in 100% (vol/vol) Me₃SO; in these experiments, the final concentration of Me₃SO, when used with PZ 51 was 5 mM, which was below the protective threshold for this agent. Results represent the mean ± SEM of three to six experiments for each drug with every scavenger; significance was determined by using the two-tailed t test for independent means. P values are indicated for the comparison between clonogenic cell survival after treatment with the anticancer quinone in the presence and the absence of a reactive oxygen scavenger. Conc., concentration; U, units.

* Catalase and SOD were heat-inactivated (HI) by autoclaving for 60 min.

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<table>
<thead>
<tr>
<th>Reactive oxygen scavengers</th>
<th>Control colonies % of control colonies</th>
<th>Me₃SO</th>
<th>Thiourea</th>
<th>Diethylurea</th>
<th>Urea</th>
<th>PZ 51</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 mM</td>
<td>60 ± 10 ± 3</td>
<td>69 ± 3</td>
<td>98 ± 6</td>
<td>84 ± 10</td>
<td>43 ± 4</td>
<td>86 ± 4</td>
</tr>
<tr>
<td>450 mM</td>
<td>63 ± 10 ± 3</td>
<td>65 ± 10</td>
<td>94 ± 4</td>
<td>64 ± 2</td>
<td>52 ± 3</td>
<td>83 ± 3</td>
</tr>
<tr>
<td>2-ml</td>
<td>104 ± 10 ± 3</td>
<td>71 ± 4</td>
<td>86 ± 3</td>
<td>87 ± 3</td>
<td>48 ± 3</td>
<td>94 ± 6</td>
</tr>
</tbody>
</table>

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* Catalase and SOD were heat-inactivated (HI) by autoclaving for 60 min.
more resistant to DOX toxicity (26). However, recent investigations have demonstrated no amelioration of DNA strand breakage by Me$_2$SO in cells exposed to DOX (27). Furthermore, urea, a structural analog of thiourea and diethylurea that is an ineffective ·OH trapping agent (23), produced no significant effect on anticancer quinone cytotoxicity. These results, taken together, support the hypothesis that ·OH formation contributed to Ehrlich tumor cell killing in the above experiments.

Catalase and SOD, scavengers of H$_2$O$_2$ and superoxide anion, respectively, significantly inhibited tumor cell killing; since the heat-denatured proteins were not protective, it is unlikely that these results were simply due to a nonspecific protein effect or to alterations in drug uptake by the antioxidant enzymes. Furthermore, over a wide range of drug concentrations, catalase significantly inhibited tumor cell killing for MMC, AZQ, and DOX (Fig. 1). It is of some interest that the drug concentrations found to inhibit clonogenic cell survival by 50% (IC$_{50}$; Table 1) approximate quite closely the peak plasma concentrations of these agents after i.v. administration in man (13, 28, 29). At the IC$_{50}$ or lower, catalase produced substantial protection against cell killing; at higher drug concentrations, the extracellular antioxidant enzyme inhibited cytotoxicity to a lesser, albeit significant, extent (Fig. 1). Inhibition of drug-related cytotoxicity by catalase occurred even though no evidence was found for the uptake of catalase into Ehrlich tumor cells in these experiments (data not shown). These studies suggest that the anticancer quinones may kill tumor cells by multiple mechanisms; however, H$_2$O$_2$-mediated events could predominate at lower drug concentrations.

As shown in Table 1, PZ 51, a lipophilic organoselenium compound that possesses glutathione peroxidase-like activity for intact cells and that can effectively detoxify H$_2$O$_2$ in the presence of glutathione (17, 18), significantly reduced the cytotoxicity of the anticancer quinones for Ehrlich cells. As a cytoprotective agent, PZ 51 was superior to catalase with respect to cell killing by DOX and AZQ.

In addition to scavengers of ·OH and H$_2$O$_2$, three iron chelating agents were examined to determine whether they could affect tumor cell killing by anticancer quinones (Table 2). Each of these agents complexes iron in a form that is unavailable for participation in reactions generating ·OH (30, 31); however, DTPA is incapable of penetrating the cell surface (32), bipyridine enters cells freely (31), and deferoxamine enters only certain mammalian cells but is capable of associating closely with the plasma membrane (33-35). Deferoxamine and bipyridine reduced the toxicity of MMC, AZQ, and DOX, whereas DTPA did not reduce Ehrlich tumor cell killing by AZQ (Table 2). These experiments suggest that the site of interaction between iron and the anticancer quinone (intracellular, extracellular, or cell membrane) may vary from drug to drug. However, since the level of iron in the tissue culture media (4 μM), which could also be present in vivo under certain conditions (36), has been demonstrated to be sufficient to catalyze ·OH production in the presence of H$_2$O$_2$ (36), these experiments support the premise that iron-related reactive oxygen metabolism may be involved in tumor killing by anticancer quinones. Finally, none of the ·OH scavengers, antioxidant enzymes, or iron chelators tested in Tables 1 and 2 produced any significant effect on the cytotoxicity of 5-iminodaunorubicin (IC$_{50}$ = 4 μM), an anthracycline analog that does not generate free radicals (data not shown). Further, the cytotoxicity of AZQ was not inhibited by SOD (Table 1). Thus, the antioxidant proteins and chemicals used in this study did not produce a general, nonspecific inhibition of drug-related tumor cell killing in these experiments.

**Effect of Anticancer Quinones on ·OH Production by Ehrlich Carcinoma Cells.** To explain the effect of ·OH scavengers and antioxidant proteins on anticancer quinone cytotoxicity, ·OH formation after treatment of intact cells with these drugs was measured. In previous studies from this laboratory, MMC, AZQ, and DOX but not 5-iminodaunorubicin have been shown to stimulate cyanide-resistant oxygen consumption by intact Ehrlich tumor cells and superoxide anion formation by Ehrlich tumor microsomes, mitochondria, and nuclei (13-15). By using a well-described assay for ·OH [or an oxidant

### Table 2. Effect of iron chelating agents on Ehrlich carcinoma cell kill by anticancer quinones

<table>
<thead>
<tr>
<th>Anticancer quinone</th>
<th>Iron chelator, % of control colony survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc., µM</td>
</tr>
<tr>
<td>MMC</td>
<td>2.5</td>
</tr>
<tr>
<td>AZQ</td>
<td>7.5</td>
</tr>
<tr>
<td>DOX</td>
<td>1.5</td>
</tr>
</tbody>
</table>

These experiments were performed as described in the legend to Table 1; results represent the mean ± SEM of three to six experiments for each drug with and without catalase pretreatment. Results are indicated for the comparison between clonogenic cell survival after treatment with the anticancer quinone in the presence and the absence of an iron chelating agent. Conc., concentration.

*$P < 0.05$.

1$P < 0.01$.

![Fig. 1. Effect of catalase on the cytotoxicity of MMC (Left), DOX (Center), and AZQ (Right) for Ehrlich carcinoma cells. Results represent the mean ± SEM of three to six experiments for each drug dose with and without catalase pretreatment. For each drug, catalase significantly reduced cytotoxicity (at least $P < 0.05$) for every dose tested.](image-url)
species with identical chemical characteristics (23, 24), it was found that the agents capable of inhibiting tumor cell killing by anticancer quinones significantly decreased ·OH production by intact, drug-treated cells (Table 3). Overall, thiourea was the most powerful inhibitor studied; this may be explained by the ability of this compound to react with and detoxify H$_2$O$_2$ and ·OH (37). In these experiments, no ·OH was detected in the absence of an anticancer quione; ·OH production was abolished similarly by heat-denaturation of the tumor cells by heat (n = 3). P values are indicated for the comparison of methane production in the presence and the absence of the reactive oxygen scavenger. Conc., concentration; U, units.

<table>
<thead>
<tr>
<th>Reactive oxygen scavenger</th>
<th>Conc. or activity</th>
<th>MMC, 250 µM</th>
<th>AZQ, 50 µM</th>
<th>DOX, 250 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>97 ± 9</td>
<td>120 ± 7</td>
<td>98 ± 6</td>
</tr>
<tr>
<td>Catalase</td>
<td>3000 U/ml</td>
<td>54 ± 3*</td>
<td>36 ± 18*</td>
<td>0 ± 0*</td>
</tr>
<tr>
<td>HI catalase</td>
<td>3000 U/ml</td>
<td>77 ± 13</td>
<td>130 ± 45</td>
<td>87 ± 2</td>
</tr>
<tr>
<td>SOD</td>
<td>50 µg/ml</td>
<td>58 ± 2*</td>
<td>120 ± 2</td>
<td>29 ± 29*</td>
</tr>
<tr>
<td>HI SOD</td>
<td>50 µg/ml</td>
<td>76 ± 2</td>
<td>90 ± 7</td>
<td>89 ± 2</td>
</tr>
<tr>
<td>Thiourea</td>
<td>200 mM</td>
<td>21 ± 21*</td>
<td>39 ± 19*</td>
<td>29 ± 29*</td>
</tr>
<tr>
<td>Urea</td>
<td>200 mM</td>
<td>76 ± 2</td>
<td>100 ± 9</td>
<td>88 ± 2</td>
</tr>
<tr>
<td>Deferoxamine</td>
<td>30 µM</td>
<td>21 ± 21*</td>
<td>62 ± 3*</td>
<td>21 ± 21*</td>
</tr>
</tbody>
</table>

Results are the mean ± SEM of three to five experiments. No methane was detected in the absence of the anticancer quinones (n = 6) or in the presence of MMC, AZQ, or DOX after denaturation of the tumor cells by heat (n = 3). P values are indicated for the comparison of methane production in the presence and the absence of the reactive oxygen scavenger. Conc., concentration; U, units.

*P < 0.05.

Table 3. Effect of anticancer quinones on ·OH production by Ehrlich carcinoma cells

Given the presence of ·OH and H$_2$O$_2$, previous experiments with DOX (9), MMC (10), or AZQ (39) have focused on the possibility that oxidation-reduction cycling of these anticancer quinones could produce DNA strand scission. However, the similarity between cytotoxicity studies and experiments demonstrating ·OH production strongly suggests that drug-stimulated superoxide anion, H$_2$O$_2$, and ·OH production play an important role in tumor cell killing as well as DNA damage by these structurally dissimilar anticancer quinones.

The finding that the antioxidant proteins catalase and SOD diminished anticancer quione cytotoxicity is of particular interest because enzymes of this size are unlikely to traverse the tumor cell plasma membrane (40). Prior studies have shown that the uptake of exogenous SOD by Ehrlich ascites cells is minimal (41); furthermore, in these experiments no evidence was found for the uptake of catalase into Ehrlich tumor cells in suspension culture. Since H$_2$O$_2$ is freely permeable across cell membranes, the protective effect of exogenous catalase could result from a reduction in the intracellular peroxide concentration by the extracellular enzyme. This conclusion is supported by the results of experiments using the organoselenium compound PZ 51. Since intracellular glutathione is required for this agent to break down H$_2$O$_2$ (17, 18) and since PZ 51 was superior to catalase in protecting cells against AZQ and DOX, it may be suggested that intracellular H$_2$O$_2$ production is strongly associated with the cytotoxic effect of anticancer quinones when these drugs are tested at their IC$_{50}$. However, as Powis and colleagues have shown (42), superoxide anion itself does not easily cross the tumor cell membrane, and, yet, SOD partially protected Ehrlich cells from the toxic effects of DOX and MMC. Thus, anticancer quione cytotoxicity in the present studies, as well as the recently described effects of impermeable, polymer-bound DOX (5, 6), could also involve H$_2$O$_2$ or superoxide anion produced at or near the tumor cell surface as well as intracellularly.

The mechanism of ·OH production by anticancer quinones has not been addressed directly in these studies. Prior investigations, however, suggest that cyclical reduction and oxidation of the quinine moiety of these drugs is involved in providing sufficient H$_2$O$_2$ for ·OH production to proceed (7-10). In fact, the precise chemical pathway of ·OH formation may differ between the drugs investigated in these experiments. However, ·OH production in each case appeared to involve the interaction of an iron catalyst with H$_2$O$_2$. The source and biochemical form of the iron involved in these experiments is still a matter of conjecture. Recent studies have shown that Ehrlich cells contain ~100 µM iron that is essentially all in protein-bound form (32). However, since DTPA and deferoxamine reduced the cytotoxicity of MMC and DOX, the 4 µM extracellular iron in the tissue culture media may itself have been the proximate source of the iron catalyst in these studies. Results with SOD, deferoxamine, DTPA, and bipyridine suggest that either the iron-catalyzed Haber-Weiss reaction (30) or a direct interaction between the MMC or DOX semiquinone and H$_2$O$_2$ to form ·OH in the presence of iron (9) could explain the findings with these drugs; the mechanism of AZQ-induced ·OH

**DISCUSSION**

It has been shown in these experiments that the cytotoxicity of DOX, MMC, and AZQ, three clinically important quinone-containing anticancer agents, can be reduced or abolished by chemicals and antioxidant proteins that detoxify or limit the formation of the ·OH and H$_2$O$_2$. Previous experiments with DOX (9), MMC (10), or AZQ (39) have focused on the possibility that oxidation-reduction cycling of these anticancer quinones could produce DNA strand scission. However, the similarity between cytotoxicity studies and experiments demonstrating ·OH production strongly suggests that drug-stimulated superoxide anion, H$_2$O$_2$, and ·OH production play an important role in tumor cell killing as well as DNA damage by these structurally dissimilar anticancer quinones.

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formation, although involving H₂O₂, may be more difficult to define.

Whatever the precise mechanism involved, the oxidizing power of the ·OH radical (43) could exert a powerful oxidant stress at multiple cellular sites in tumors treated with quinone-containing antineoplastic drugs. The potentially lethal events that could be produced by H₂O₂ or ·OH in tumor cells are numerous but include deleterious effects on cellular calcium homeostasis (44) and energy production (45) as well as diminished reproductive potential (31).

The real possibility that oxygen free radicals are involved in the therapeutic action of the anticancer quinones MMC, AZQ, and DOX suggests, finally, that strategies aimed at altering the defenses of the tumor cell against oxidation may be a novel method of enhancing the effectiveness as well as the spectrum of activity for this widely used class of antineoplastic compounds.

This study was supported by Grant CA 31788 from the National Cancer Institute and by the Leukemia Society of America.