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Nicotinamide Adenine Dinucleotide (Phosphate):Quinone Oxidoreductase (DT-Diaphorase) as a Target for Bioreductive Antitumor Quinones: Quinone Cytotoxicity and Selectivity in Human Lung and Breast Cancer Cell Lines

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SUMMARY

Bioreductive antitumor quinones require reductive metabolism to produce their cytotoxic effects. A series of these compounds was screened for relative rates of reduction by the two-electron reductase, NAD(P)H:quinone oxidoreductase (DTD). The antitumor quinones streptonigrin (SN), 2,5-diaziridinyl-3-phenyl-1,4-benzoquinone (PDZQ), 2,5-diaziridinyl-3,6-dimethyl-1,4-benzoquinine (MeDZQ), and [3-hydroxymethyl-5-aziridinyl-1-methyl-2-(1*H*-indole-4,7-dione)-propenol] (EO9) were all excellent substrates for recombinant rat and human DTD. All four compounds were reduced by DTD at least 100 times faster than the clinically important bioreductive alkylating agent, mitomycin C (MC). Reduction of the antitumor quinones was generally 4–5 times more efficient by rat DTD than by human DTD. The exception was EO9, which, surprisingly, was reduced 23 times faster by rat DTD than by human DTD. The rate of reduction of each individual quinone was similar under either aerobic or anaerobic conditions, suggesting that DTD may be an important activating enzyme in the hypoxic fraction of solid tumors. The cytotoxicity of MeDZQ and MC was examined in a panel of human breast and lung cancer cell lines. The data showed good correlations between DTD activity and toxicity for both MeDZQ ($r = 0.57$, $p = 0.054$) and MC ($r = 0.69$, $p = 0.020$), confirming biochemical data that both compounds are bioactivated by DTD. In addition, IC_{50} values were in general lower for MeDZQ than for MC in cell lines containing elevated DTD, a finding that was consistent with metabolic data that indicated that MeDZQ was a better substrate for DTD than MC. SR, defined as the ratio of the IC_{50} value for the H596 NSCLC cell line (undetectable DTD activity) to the IC_{50} value for the H460 NSCLC cell line (high DTD activity), were determined for all five antitumor quinones. SN was the most selective (SR = 86) followed by EO9 (SR = 62), MeDZQ (SR = 17), and MC (SR = 11). Surprisingly, PDZQ, an excellent substrate for DTD, was toxic to both cell lines (SR = 1.8). These data suggest that antitumor quionones that are substrates for DTD may be selectively toxic to tumors with high DTD activity and may be useful in the treatment of those tumors.

The goal of cancer chemotherapy is to use cytotoxic agents that are selectively toxic to cancer cells with minimal toxicity to normal tissues. One approach to achieving selective toxicity is to target enzymes that are overexpressed in cancer cells relative to normal cells; this is an enzyme-directed approach to chemotherapy (1). A potential target enzyme is DTD (EC 1.6.99.2), an obligate two-electron reductase that is characterized by its capacity for using either NADH or NADPH as reducing cofactors and by its inhibition by dicumarol (2). DTD can protect the cell from a broad range of chemically reactive metabolites (3) and it is commonly recognized as a detoxification enzyme with respect to quinone metabolism (4, 5).

DTD can also function as an activating enzyme, specifically for the reductive activation of antitumor quinones and other bioreductive antitumor compounds. Various reports have demonstrated that MC (6), AZQ (7) and its analogues (8), EO9 (9), and the dinitrophenylaziridine CB1954 (10) are bioactivated by DTD. In the course of testing a number of bioreductive antitumor compounds, we have identified several that are much more efficient substrates for DTD than MC, a clinically important bioreductive alkylating agent (11). In addition, when a series of AZQ analogues was tested for

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ABBREVIATIONS: DTD, NAD(P)H:quinone oxidoreductase; DCPIP, 2,6-dichlorophenol-indophenol, SN, streptonigrin; MC, mitomycin C; EO9, 3-hydroxymethyl-5-aziridinyl-1-methyl-2-(1*H*-indole-4,7-dione)-propenol; MeDZQ, 2,5-diaziridinyl-3,6-dimethyl-1,4-benzoquinone; PDZQ, 2,5-diaziridinyl-3-phenyl-1,4-benzoquinone; AZQ, diaziquone; DZQ, 2,5-diaziridinyl-1,4-benzoquinone; HPLC, high performance liquid chromatography; MTT, 3-(4,5-dimethylthiazol-2,5-diphenyl)tetrazolium; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; IC_{50}, concentration at which cell survival equals 50% of control; SR, selectivity ratio; PBS, phosphate buffered saline.

their cytotoxicity in HT-29 (DTD-rich) and BE (DTD-deficient) human colon carcinoma cell lines, a correlation was observed between rates of reduction by purified DTD and cytotoxicity to HT-29 cells (12). Two of the compounds, DZQ and MeDZQ, were much better substrates for DTD than AZQ and were 1 to 2 orders of magnitude more cytotoxic to HT-29 cells than AZQ based on IC_{90} values. DZQ and MeDZQ were also 5–6 times more toxic to the DTD-rich HT-29 cells than to the DTD-deficient BE cells, indicating that DTD may be important in the activation of bioreductive antitumor compounds in DTD-rich tumor cells (12).

Marked elevations in DTD activity and mRNA content have been documented in both preneoplastic tissues and established tumors (3). Tumors or cancer cell lines in which increased DTD expression has been observed include those from the lung (13, 14), liver (13, 15), colon (13), breast (13, 16), and brain (17). Schlager and Powis (13) measured DTD activity in cytosol from 17 paired human primary breast tumors and normal tissue. They found that DTD activity was elevated 3-fold in breast tumor tissue relative to normal tissue (13). Workman *et al.* (16) have also reported that DTD is overexpressed in breast tumors. In addition, we have shown that human NSCLCs have markedly elevated DTD activity relative to SCLCs and normal tissue (14). These reports suggest that breast cancer and NSCLC may be relevant targets for bioreductive antitumor compounds that are efficiently activated by DTD.

NSCLC accounts for the majority of lung cancers, yet the prognosis in advanced cases remains poor. MC is the prototypical bioreductive alkylating agent and has been shown to be the most active agent for the treatment of NSCLC (18). Severe bone marrow suppression and gastrointestinal toxicity accompanying the systemic use of MC alone or in combination, however, have limited its clinical usefulness. In the present study, we examined the relationship between DTD activity in human breast and lung cancer cell lines and the metabolism and cytotoxicity of a series of bioreductive antitumor quinones. We also used cell lines with either high or low DTD activity in an attempt to define the selectivity of compounds for tumors with elevated DTD.

Materials and Methods

Chemicals. MeDZQ was synthesized by the method of Cameron and Giles (19). MC was obtained from the Pharmaceutical Research and Development Division, Bristol-Myers Squibb Co. (Syracuse, NY). EO9 was provided by Dr. Jeff Cummings (Imperial Cancer Research Fund, Edinburgh, UK). SN, DCPIP, and NADH were obtained from Sigma Chemical Co. (St. Louis, MO). Dicumarol was obtained from Aldrich Chemical Co. (Milwaukee, WI). All other reagents were of analytical grade or better.

Preparation of PDZQ. Phenyl-1,4-benzoquinone (1 g, 5.4 mmol) was dissolved in dry ethanol (50 ml); this solution was then cooled on ice. Aziridine (0.85 ml, 16.4 mmol) was dissolved in dry ethanol (1 ml) and also cooled on ice. The aziridine/ethanol solution was then added to the quinone solution and was stirred for 1.5 h. The resulting precipitate was filtered and washed with cold dry ethanol. The product was then recrystallized from dry methanol (376 mg, 26% m.p., 163.5–164.5°).[^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.35 (m, 5 H, Ph), 6.05 (s, 1 H, C_6H), 2.28 (s, 4 H, Az-1), 2.02 (s, 4 H, Az-2); MS (EI) m/z: 266(M^+), 265, 251, 237, 210, 115, 67; λ_{max}(EtOH): 220, 336, 460 (no base shift) nm.]

Cell culture. All cell lines were grown in minimum essential medium with Earle's salts, nonessential amino acids, L-glutamine, and penicillin/streptomycin and supplemented with 10% fetal bovine serum. T47D cells were also supplemented with 1 nM insulin. Cell culture medium and supplements were obtained from Life Technologies, Inc. (Grand Island, NY). The cells were incubated at 37° under a humidified atmosphere containing 5% CO_2.

Rat and human recombinant DTD. Rat and human recombinant DTD from *Escherichia coli* were expressed, purified, and characterized as previously described (11).

Spectrophotometric analysis. Reduction of MeDZQ, EO9, and SN was followed by UV/visible spectrophotometry with a Hewlett-Packard Model HP8452 diode-array spectrophotometer. Reactions contained 320 μM NADH, either MeDZQ, EO9 or SN, rat or human recombinant DTD, and 25 mM Tris·HCl, pH 7.4, plus 2 mg Tween 20/ml. NADH oxidation was followed at 292 nm (MeDZQ), 338 nm (EO9), or 362 nm (SN). At least three reactions were run at each concentration of MeDZQ, EO9, and SN. K_m and k_{cat} values were determined using the Enzfitter data analysis program (Biosoft, Cambridge, UK).

HPLC analysis. Reduction of the antitumor quinones was followed by HPLC with an Alltech C18 (5 μm, 250 × 4.6 mm) column with a Shimadzu HPLC system (SCL-6A controller, SPD-6AV UV-Vis detector, two LC-6A pumps, and a C-R3A integrator). The solvent program used a linear gradient of 5–80% B for 10 min, 80% B for 5 min, then 80–5% B for 5 min (solution A: 10 mM potassium phosphate buffer, pH 6.0; solution B: methanol). Reactions were run in 25 mM Tris·HCl, pH 7.4, except for MC (pH 5.8, with 200 μM NADH, 50 μM antitumor quinone, and rat or human recombinant DTD). NADH oxidation and antitumor quinone removal were quantified at 340 nm after 30-min incubations at 22°. For anaerobic reactions, solutions were continuously gassed with prepurified Argon.

Oxygen utilization. Oxygen uptake was measured in 3-ml reactions with a Clarke-type electrode (Yellow Springs Instrument Co., Yellow Springs, OH) at 22° with air-saturated water for calibration. Oxygen content of air-saturated water was adjusted for temperature and altitude.

DTD activity assay. DTD activity in the cancer cell lines was determined spectrophotometrically by monitoring DCPIP reduction at 600 nm (2). Cells were grown to 75–80% confluence, trypsynized, washed in PBS, and resuspended in 25 mM Tris·HCl/250 mM sucrose, pH 7.4. Cell suspensions were sonicated on ice and assayed immediately. Protein was determined by the method of Lowry *et al.* (20).

Cytotoxicity assay. Cytotoxicity was determined with the MTT colorimetric assay (21). Cells were plated in 96-well plates at a density of 1–2 × 10^4 cells/ml and allowed to attach overnight (16 hr). Drug solutions were applied in serumless medium for 2 hr. Drug solutions were removed and replaced with complete medium (with serum), and the 96-well plates were incubated for 5–7 days. MTT (50 μg) was added, and the cells were incubated for an additional 4 hr. Medium/MTT solutions were removed carefully by aspiration, the MTT formazan crystals were dissolved in 100 μl DMSO, and absorbance was determined on a plate reader at 550 nm. IC_{50} values were determined from semilog plots of percent of control versus concentration.

Results

The *in vitro* metabolism of five bioreductive antitumor quinones (Fig. 1) by rat and human recombinant DTD was compared. For EO9, MeDZQ, and SN, values for K_m, k_{cat}, and k_{cat}/K_m were determined with UV/visible spectroscopy and are presented in Table 1. Consistent with our earlier report (11), reduction by rat recombinant DTD was in general more efficient than reduction by human recombinant DTD. All three antitumor quinones were excellent substrates for the rat enzyme based on their respective k_{cat} values. A comparison of k_{cat}/K_m values (enzymatic efficiency at low substrate concentrations) suggests that EO9 is the best substrate for

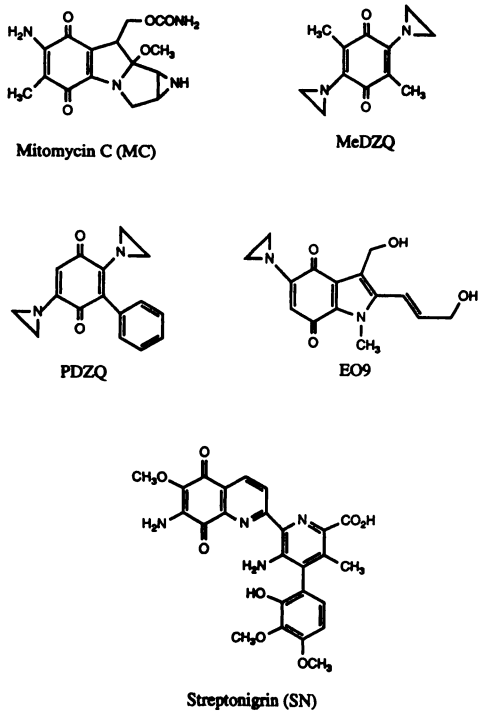

Fig. 1. Structures of bioreductive antitumor quinones.

TABLE 1

Kinetics of MeDZQ, EO9, and SN reduction by rat and human recombinant DTD

Reactions contained 320 μM NADH, MeDZQ, EO9 or SN, rat or human recombinant DTD, and 25 mM Tris · HCl, pH 7.4, plus 2 mg Tween 20/ml. NADH oxidation was followed at 292 nm (MeDZQ), 338 nm (EO9), or 362 nm (SN). At least three reactions were run at 22° for each concentration of MeDZQ, EO9, or SN.

	K_M	k_{cat}	k_{cat}/K_M
	μM	min^{-1}	$\mu M^{-1}/min^{-1}$
MeDZQ			
Rat DTD	109 ± 18	17,700 ± 2,700	162
Human DTD	33.2 ± 16.8	3,940 ± 650	119
EO9			
Rat DTD	22.3 ± 2.7	21,700 ± 1,100	973
Human DTD	13.6 ± 1.9	778 ± 34	57.2
SN			
Rat DTD	53.3 ± 12.6	23,100 ± 2,500	433
Human DTD	35.6 ± 1.7	7,320 ± 250	206

Values are mean ± standard deviation for a minimum of three determinations.

rat DTD, followed in order by SN and MeDZQ. A different result was observed with human DTD. For SN and MeDZQ, k_{cat} values for human DTD were 25–30% of the values obtained with rat DTD, as expected. For EO9, however, this value was less than 4%. A comparison of k_{cat}/K_m values in this case showed that SN was the best substrate for human DTD, followed by MeDZQ and then EO9.

A comparison of reduction velocity by rat and human recombinant DTD for all five antitumor quinones at a fixed substrate concentration with the use of HPLC is presented in Table 2. SN, PDZQ, MeDZQ, and EO9 were reduced by rat recombinant DTD at a rate that was 2–3 orders of magnitude greater than the reduction rate for MC, and the same trend was observed with human recombinant DTD. Consistent with the data in Table 1, EO9 was again found to be a better substrate for the rat enzyme than MeDZQ but was reduced 3–4 times slower than MeDZQ by human DTD.

Antitumor quinone metabolism was also compared under

TABLE 2

Metabolism of antitimor quinones by rat and human recombinant DTD

Reactions contained 200 μM NADH, 50 μM antitumor quinone, rat or human recombinant DTD, and 25 mM Tris · HCl, pH 7.4, with the exception of MC, pH 5.8. NADH oxidation was quantified by HPLC at 340 nm after 30-min incubations at 22°.

	Velocity NADH oxidation
	μmol/min/mg
MeDZQ	
Rat DTD	104 ± 31
Human DTD	25 ± 4
EO9	
Rat DTD	181 ± 19
Human DTD	7.7 ± 2.0
SN	
Rat DTD	243 ± 42
Human DTD	51 ± 4
PDZQ	
Rat DTD	228 ± 44
Human DTD	60 ± 5
MC	
Rat DTD	0.68 ± 0.02
Human DTD	0.15 ± 0.01

Values are mean ± standard deviation for a minimum of three reactions.

aerobic versus anaerobic conditions using rat recombinant DTD. Table 3 lists velocities for NADH oxidation and antitumor quinone removal under aerobic and anaerobic conditions and oxygen uptake under aerobic conditions for the five antitumor compounds. In general, reduction of each individual quinone by DTD was similar under aerobic or anaerobic conditions because there were only minor differences in NADH oxidation with each compound. This is in agreement with our earlier work with MC (11). Under aerobic conditions, the amount of oxygen uptake observed was approximately equal to the difference between NADH oxidation and antitumor quinone removal for each compound, as predicted. The high rate of oxygen uptake and low rate of antitumor quinone removal observed for SN, PDZQ, and EO9 imply that these compounds are rapidly reoxidized in air after reduction by DTD. In comparison, MeDZQ and MC demonstrated markedly less redox cycling capability, with oxygen uptake at 33% and 18%, respectively, of NADH oxidation under aerobic conditions. The anaerobic data showed that NADH oxidation and antitumor quinone removal were virtually stoichiometric for MeDZQ and MC and were similar for PDZQ. This was the expected result because oxygen was removed from the system, thereby eliminating the potential for redox cycling. However, SN and EO9 metabolism under anaerobic conditions did not give the expected stoichiometry. This was likely due to the use of an analytical HPLC system that was not oxygen free and to the rapid reoxidation of these two compounds in air.

The relationship between DTD activity and antitumor quinone cytotoxicity in cancer cell lines was studied in five lung cancer cell lines (Table 4) and four breast cancer cell lines (Table 5) with MC and MeDZQ. MC and MeDZQ were more toxic to cell lines with high or moderate DTD activity than to cell lines with low or undetectable DTD activity. When these data were plotted on semilog graphs of IC_{50} versus DTD activity for all nine cell lines, correlations were observed for MC (Fig. 2A, $r = 0.69$, one-tailed $p = 0.02$) and MeDZQ (Fig. 2B, $r = 0.57$, one-tailed $p = 0.05$). An additional finding was that MeDZQ was more toxic than MC in cell lines with

TABLE 3

Metabolism of antitumor quinones by rat recombinant DTD under aerobic and anaerobic conditions

Reactions contained 200 μM NADH, 50 μM antitumor quinone, rat recombinant DTD, and 25 mM Tris · HCl, pH 7.4, with the exception of MC, pH 5.8. NADH oxidation and antitumor quinone removal were quantified by HPLC at 340 nm after 30-min incubations at 22°. Solutions were made anaerobic by continuous gassing with prepurified Argon. Oxygen uptake was quantified over a 30-min period at 22° and was adjusted for temperature and altitude.

	Velocity		
	NADH oxidation	Antitumor quinone removal	Oxygen uptake
	μmol/min/mg		
MeDZQ			
Aerobic conditions	104 ± 31	78 ± 34	33 ± 10
Anaerobic conditions	104 ± 37	92 ± 32	—
EO9			
Aerobic conditions	181 ± 19	8.6 ± 4.3	222 ± 10
Anaerobic conditions	159 ± 21	27 ± 7	—
SN			
Aerobic conditions	243 ± 42	ND	290 ± 8
Anaerobic conditions	173 ± 26	11 ± 6	—
PDZQ			
Aerobic conditions	228 ± 44	37 ± 29	211 ± 3
Anaerobic conditions	186 ± 13	147 ± 20	—
MC			
Aerobic conditions	0.68 ± 0.02	0.51 ± 0.03	0.12 ± 0.01
Anaerobic conditions	0.48 ± 0.05	0.47 ± 0.01	—

Values are mean ± standard deviation for a minimum of three reactions.
ND = not detectable, no difference from controls.

TABLE 4

DTD activity in lung cancer cell lines and cytotoxicity of MeDZQ and MC to lung cancer cell lines

DTD activity was determined by monitoring DCPIP reduction at 600 nm with cell sonicates. IC_{50} values were determined from semilog plots of percent of control versus concentration with the MTT colorimetric assay.

Cell line/ drug	DTD activity	IC_{50}
	nmol/min/mg	*μM*
H460	1360 ± 100[a]	
MC		0.21 ± 0.03[b]
MeDZQ		0.043 ± 0.009
H520	1090 ± 130	
MC		0.19 ± 0.06
MeDZQ		0.063 ± 0.004
H125	542 ± 93	
MC		0.35 ± 0.04
MeDZQ		0.049 ± 0.010
H596	ND	
MC		2.3 ± 0.8
MeDZQ		0.71 ± 0.31
H446	ND	
MC		1.2 ± 0.4
MeDZQ		1.4 ± 0.3

[a] Mean ± standard deviation for six separate experiments.
[b] Mean ± standard deviation for a minimum of three separate experiments.
ND = not detectable, <5 nmol/min/mg.

elevated DTD activity. This was consistent with the metabolic data that showed that MeDZQ was a much better substrate for DTD than MC.

To study the selectivity of all five antitumor quinones, the two NSCLC cell lines with the highest (H460) and lowest (H596) DTD activities were chosen. Cytochrome P-450 reductase (measured as NADPH-cytochrome *c* reductase) and cytochrome b_5 reductase (measured as NADH-cytochrome *c* reductase) levels were also measured in the H460 and H596 cell lines. Cytochrome P450 and cytotchrome b_5 reductase activities were higher in H596 cells (13 and 17 nmol/min/mg, respectively) than in H460 cells (5.0 and 7.9 nmol/min/mg, respectively). We defined a selectivity ratio as the IC_{50} value for the H596 NSCLC cell line (undetectable DTD activity) divided by the IC_{50} value for the H460 NSCLC cell line (DTD activity = 1360 nmol/min/mg). These results are presented in Table 6. SN was the most selective, followed by EO9, MeDZQ, and MC in that order. Surprisingly, PDZQ, an excellent substrate for DTD, was only ~2 times more toxic to DTD-rich H460 cells than to DTD-deficient H596 cells.

Discussion

We studied the *in vitro* metabolism of five bioreductive antitumor quinones by DTD and the toxicity of those compounds in breast and lung cancer cell lines with a wide range of DTD activities. Our results suggest that bioreductive antitumor quinones that are good substrates for DTD are selectively toxic to cancer cell lines with elevated DTD activity.

The results of the rat and human DTD metabolism studies are consistent with data we have published previously (11). Rates of reduction by human recombinant DTD in both studies were between 20% and 50% of the reduction rates by rat recombinant DTD. An exception to this trend was found during EO9 metabolism. EO9 was an excellent substrate for rat DTD but was reduced by the human enzyme at a rate of only ~4% of the rate observed for the rat enzyme. The reason for this is unclear, but both the kinetic and HPLC studies confirmed the lower rate for human DTD. Nevertheless, EO9 was a much better substrate for human DTD than the clinical agent MC.

We examined aerobic and anaerobic metabolism of the bioreductive antitumor agents by DTD because these agents were originally developed for use in solid tumors containing a fraction of hypoxic cells (22). Thus, it was important to determine whether these compounds were activated by DTD under oxygen-deficient conditions as well as aerobic conditions. NADH oxidation was similar under aerobic and anaerobic conditions for all five compounds, indicating that DTD functions equally in the presence or absence of oxygen. This suggests that DTD is a reasonable target for enzyme-directed therapy of hypoxic tumors with elevated DTD activity. Sup-

TABLE 5

DTD activity in breast cancer cell lines and cytotoxicity of MeDZQ and MC to breast cancer cell lines

DTD activity was determined by monitoring DCPIP reduction at 600 nm with cell sonicates. IC_{50} values were determined from semilog plots of percent of control versus concentration with the MTT colorimetric assay.

Cell line/drug	DTD activity	IC_{50}
	nmol/min/mg	*μM*
MCF7	564 ± 95[a]	
MC		0.35 ± 0.05[b]
MeDZQ		0.044 ± 0.006
MDA231	406 ± 58	
MC		0.25 ± 0.05
MeDZQ		0.073 ± 0.024
ZR75	354 ± 24	
MC		0.65 ± 0.20
MeDZQ		0.083 ± 0.019
T47D	68 ± 23	
MC		0.87 ± 0.11
MeDZQ		0.094 ± 0.006

[a] Mean ± standard deviation for six separate experiments.
[b] Mean ± standard deviation for a minimum of three separate experiments.

port for this hypothesis can be found in our recent report (11), in which we showed that MC was equally toxic to DTD-rich HT-29 human colon carcinoma cells under aerobic or hypoxic conditions. Overall, SN, PDZQ, and EO9 were the best substrates for rat DTD, whereas SN, PDZQ, and MeDZQ were the best substrates for human DTD.

In our present investigation of the relationship between DTD activity and cytotoxicity of MC and MeDZQ in lung and breast cancer cells, a good correlation was observed for both compounds. Increased DTD activity was accompanied by increased MC and MeDZQ toxicity. This result is consistent with our previous work (6, 12) but contrary to that reported by Robertson *et al.* (23) for MC. They reported no relationship between DTD activity and MC and porfiromycin (an analogue of MC) toxicity in breast, lung, and colon cell lines. The protocol of Robertson *et al.* (23) used continuous exposure to the antitumor quinone, and this may have had an effect on DTD expression during treatment. Our results are based on a 2-hr exposure to the antitumor quinone beginning at 16 hr after plating. Our data clearly show that MC cytotoxicity is significantly correlated with DTD activity, and this is consistent with other reports (reviewed in Ref. 24). In a recent report in which cytotoxicity data for >31,000 compounds were correlated with cellular DTD activity in the NCI human tumor cell line panel, MC was among the top 25 compounds (25). Consistent with our data, the number 1 compound in this study ranked by correlation coefficient was SN (25).

A correlation between DTD activity and EO9 toxicity has been reported by Robertson *et al.* (23, 26) and Smitskamp-Wilms *et al.* (27). In these reports, a panel of cell lines with varying levels of DTD activity was assessed for EO9 cytotoxicity, the same approach that we used for MC and MeDZQ. In an attempt to characterize the efficiency with which a series of quinones target DTD, we extended this approach to include a selectivity ratio. Higher selectivity ratio values are associated with greater toxicity to the high-activity DTD cell line relative to the low DTD cell line. The compound with the highest selectivity ratio, and therefore the most selective agent, was SN. Despite EO9 being a poor substrate for human DTD relative to MeDZQ, it demonstrated a more favorable selectivity ratio in these two cell lines than MeDZQ. SN is not an alkylating agent but rather an efficient redox cycling compound that is believed to act through the generation of hydroxyl radicals in the presence of Cu^{2+} and Fe^{2+}. A SN/metal/DNA complex is proposed that does not involve intercalation (28). SN was investigated clinically in the 1960s, but problems with delayed myelotoxicity precluded its widespread use (29, 30). If toxicity problems could be ameliorated or overcome, our data suggest that SN may be a useful compound with which to target DTD in tumor cells. EO9, like SN, is an efficient redox cycler, but it has multiple alkylating moieties. EO9 has shown no myelotoxicity in preclinical studies (31) and is currently in phase I and II clinical trials with the European Organization for Research and Treatment of Cancer (27). MeDZQ also showed good selectivity, as did MC, the only agent currently in widespread clinical use. MeDZQ and MC are bifunctional alkylating agents that are not readily reoxidized after reduction. The contribution of

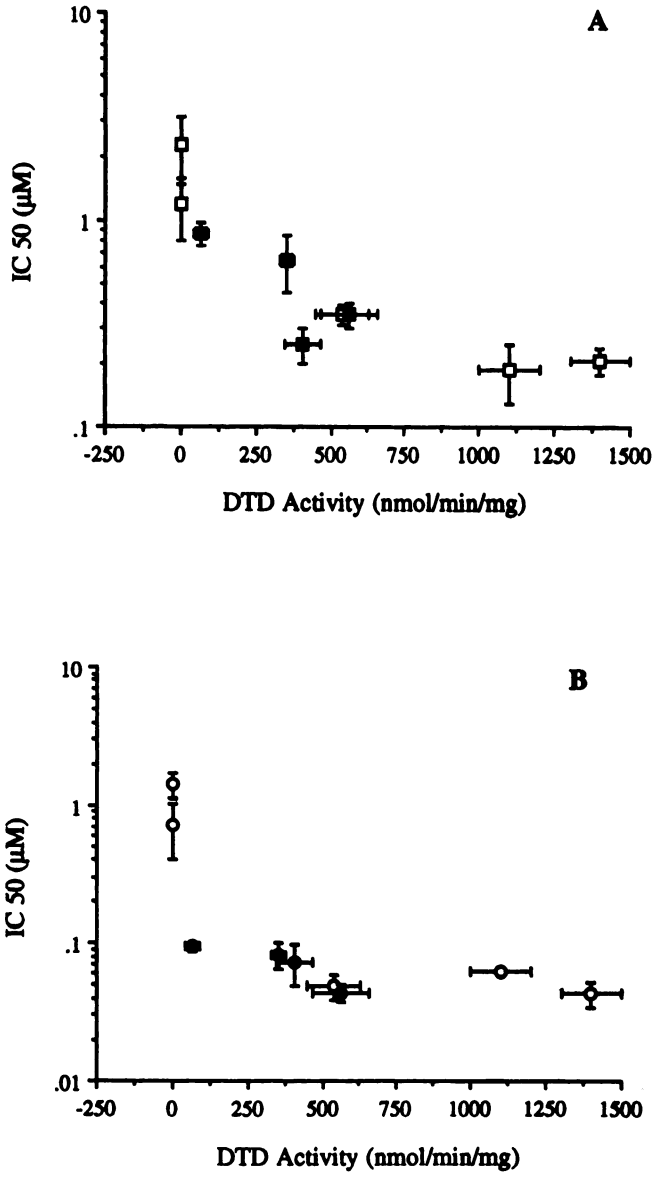

Fig. 2. Correlation between DTD activity and cytotoxicity of (A) MC (r = 0.69, p = 0.020) and (B) MeDZQ (r = 0.57, p = 0.054) in human lung and breast cancer cell lines. □, MC toxicity in lung cancer cell lines; ■, MC toxicity in breast cancer cell lines; ○, MeDZQ toxicity in lung cancer cell lines; ●, MeDZQ toxicity in breast cancer cell lines.

TABLE 6

DTD activity in lung tumor cell lines and cytotoxicity of antitumor quinones to lung tumor cell lines

DTD activity was determined by monitoring DCPIP reduction at 600 nm with cell sonicates. IC_{50} values were determined from semilog plots of percent of control versus concentration with the MTT colorimetric assay. Selectivity ratios are the IC_{50} values for the H596 cell line divided by the IC_{50} values for the H460 cell line.

Cell line/drug	DTD activity	IC_{50}	Selectivity ratio
	nmol/min/mg	*μM*	
H460	1360 ± 100[a]		
MC		0.21 ± 0.03[b]	11
MeDZQ		0.043 ± 0.009	17
SN		0.0058 ± 0.0036	86
EO9		0.029 ± 0.006	62
PDZQ		0.071 ± 0.020	1.8
H596	ND		
MC		2.3 ± 0.8	
MeDZQ		0.71 ± 0.31	
SN		0.50 ± 0.26	
EO9		1.8 ± 0.4	
PDZQ		0.13 ± 0.05	

[a] Mean ± standard deviation for six separate experiments.
[b] Mean ± standard deviation for a minimum of three separate experiments.
ND = not detectable, <5 nmol/min/mg.

redox cycling ability to selectivity is unknown because another efficient redox cycler, PDZQ, was the least selective agent of the five antitumor quinones tested in this study. The reasons underlying the lack of selectivity of PDZQ are unclear but may include alternative routes of bioactivation in H596 cells or chemical degradation.

In conclusion, a good correlation was observed between DTD activity and bioreductive antitumor quinone cytotoxicity in lung and breast cancer cell lines. With the exception of PDZQ, the best substrates for DTD showed preferential toxicity to cell lines with elevated DTD activity and therefore may be useful in the treatment of tumors with elevated DTD activity, such as breast cancer and NSCLC. Considering DTD to be a target enzyme, SN, EO9, and MeDZQ would be predicted to be more potent and more selective than the currently used clinical agent, MC.

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