Novel NAD(P)H:quinone oxidoreductase 1 (NQO1)-Directed Quinolinequinone Antitumor Drugs

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Abstract

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a two-electron reductase implicated in the bioactivation of antitumor quinones. Expressed at high levels in many human solid tumors, NQO1 is a unique target for enzyme-directed bioreductive cancer drug development. Quinone antitumor agents can be bioactivated by cancer cells that overexpress NQO1 to exhibit selective toxicity. We report on the effects of functional group changes on the toxicity of novel quinolinequinones to human colon adenocarcinoma cells with either no detectable NQO1 activity (BE-WT) or high NQO1 activity (BE-NQ). Various substituents were added to the 2, 3, and/or 6-positions of quinoline-5,8-dione. Previously, the metabolism (reduction rates) of the quinones by purified recombinant human NQO1 had been determined by a spectrophotometric assay that used cytochrome *c* as the terminal electron acceptor. Toxicity was determined by the MTT colorimetric assay. The relationship between metabolism and toxicity was evaluated for seven quinolinequinone compounds. In general, the quinolinequinones with the highest reduction rates showed the greatest selective toxicity toward the high-NQO1 BE-NQ cells. Two of the most selective compounds were HP414 and HP436. These two compounds contain pyridine substituents that can act as part of a molecular cavity with potential metal binding sites. This provides the ability to utilize metals to bind DNA and act as catalysts for the production of reactive oxygen species leading to DNA strand breaks and cytotoxicity. Supported by the NIH Grant R25ES16247.

Introduction

The goal of cancer drug discovery is to design cytotoxic compounds that selectively interact with molecular targets unique to tumor cells with minimal toxicity to normal cells. One approach to achieve selective toxicity is through bioreductive activation and identifying reductase enzymes that are over expressed in tumor cells when compared to normal cells. NAD(P)H:quinone oxidoreductase (NQO1) is a widely distributed homodimeric flavoenzyme containing one molecule of FAD prosthetic group per 31 kDa subunit. This enzyme obligate two-electron reductase is present in cytosol (> 90%) and nucleus and catalyzes a nicotinamide nucleotide-dependent two-electron reduction and the bioactivation of quinone-based chemotherapeutic compounds. Marked elevations in NQO1 activity have been documented in various tumors and suggest that NQO1 is a good candidate for enzyme directed bioreductive drug development

The cytotoxic mechanism of quinones against cancer cells is highly related to the bioreduction process for activation of the quinones. The hydroquinone produced after the two-electron reduction of anticancer quinones is the biologically active form that can cause DNA alkylation and/or oxidative stress. Quinones are powerful redox active agents that can undergo redox cycling with concomitant production of reactive oxygen species (ROS). The NQO1-mediated redox cycling of quinones can produce large quantities of ROS that can overpower the antioxidant defense system and alter the intracellular redox balance leading to severe oxidative stress. DNA has been recognized as the principal target of quinone-based anticancer compounds. ROS and in particular hydroxyl radicals are capable of attacking DNA and oxidizing guanine bases resulting in the formation of mutagenic 8-hydroxy-2'-deoxyguanosine (8-oxo-2dG) lesions and DNA strand breaks. Redox cycling-mediated oxidative stress, depletion of protective glutathione and oxidative DNA damage are important mechanisms of antitumor quinone toxicity.

Quinolinequinone compounds act as potential bioreductive substrates for NQO1 that can be bioactivated in tumors with increased levels of NQO1 and may be selectively toxic to those tumors. Some of these compounds contain pyridine or similar groups that can act as part of a molecular cavity with potential metal binding sites providing DNA binding capability and ROS catalysis. The presence of metal ions catalyzes the reaction leading to generation of hydroxyl radicals. This study was conducted to clarify the role of NQO1 in bioactivation of quinolinequinone compounds and to verify whether activation by NQO1 resulted in selective cytotoxicity.

Results

Table 1. Metabolism of quinolinequinone compounds by recombinant human NQO1 monitored by spectrophotometric cytochrome *c* assay (indicating initial reduction rate) and HPLC (indication extended reduction rate). *Reduction rates measured in umol/min/mg.*

Compound	R^2	R^3	R^6	Metabolism (µmol/min/mg) NADH oxidation (by HPLC)	Metabolism by NQO1 (µmol/min/mg) (cytochrome *c* reduction)
HP460	Me	H	OMe	20.7 ± 3.7	342 ± 22.9
TF03	Cl	H	OMe	100 ± 32.0	420 ± 40.1
TF02	Ph	H	OMe	28.8 ± 7.9	314 ± 58.8
HP436	pyridin-2-yl	H	OMe	22.6 ± 4.4	446 ± 39.0
HP414	pyridin-3-yl	H	OMe	61.9 ± 8.6	569 ± 59.7
TF04	thiophen-2-yl	H	OMe	32.1 ± 7.8	308 ± 57.1
TF07	benzofur-2-yl	H	OMe	33.3 ± 16.8	191 ± 62.9

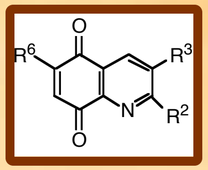
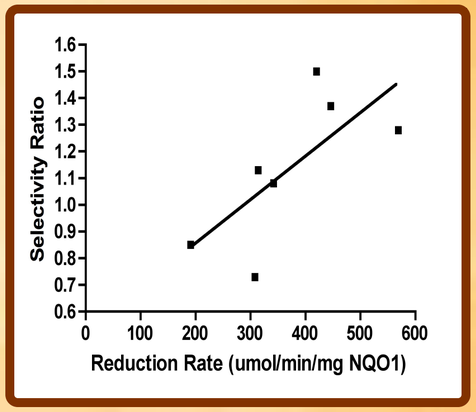


Table 2. Cytotoxicity of quinolinequinone compounds (24 hour exposure) towards BE-WT (NQO1-deficient) and BE-NQ (NQO1-rich) human colon adenocarcinoma cell lines.

Compound	R^2	R^3	R^6	IC_{50} (µM) BE-WT	IC_{50} (µM) BE-NQ	Selectivity Ratio [IC_{50}(BE-WT)/ IC_{50}(BE-NQ)]
HP460	Me	H	OMe	11.2 ± 2.3	10.4 ± 2.0	1.1
TF03	Cl	H	OMe	7.2 ± 1.1	4.8 ± 0.3	1.5
TF02	Ph	H	OMe	18.1 ± 0.3	16.0 ± 1.1	1.1
HP436	pyridin-2-yl	H	OMe	12.7 ± 3.0	9.3 ± 1.2	1.4
HP414	pyridin-3-yl	H	OMe	10.5 ± 3.2	8.2 ± 1.0	1.3
TF04	thiophen-2-yl	H	OMe	4.4 ± 0.7	6.0 ± 1.5	0.7
TF07	benzofur-2-yl	H	OMe	17.3 ± 6.3	20.3 ± 4.5	0.9

Figure 1. Correlation of the reduction rates obtained from the spectrophotometric assay and selectivity ratios obtained by dividing the IC_{50} values of BE-WT (NQO1-deficient) by the IC_{50} values of BE-NQ (NQO1-rich) cells. Linear regression data include an r^2 value of 0.5088 and a p value of 0.0719. *IC_{50} values obtained by the MTT assay.*

Trendline Equation:	y = 0.0016x + 0.5312
R^2 Value	0.5088
P Value	0.0719



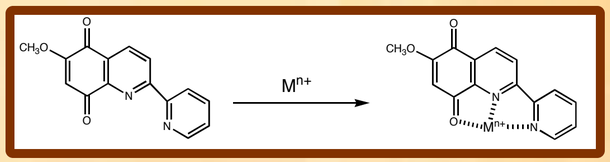


Figure 2. Molecular structure of compound HP436. Metal binding site located in the molecular cavity formed by the quinolinequinone and pyridine substituent. *Both compounds HP414 and HP436 include pyridine substituents.*

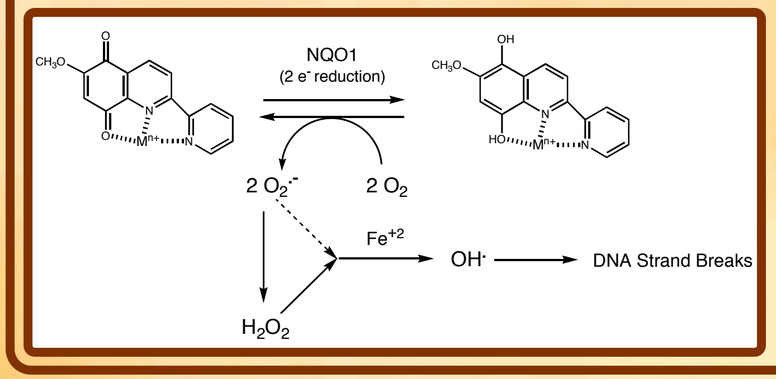


Figure 3. Cytotoxic mechanism of quinones. The hydroquinone produced after the two-electron reduction causes oxidative stress by redox cycling with concomitant production of ROS. Hydroxyl radicals are capable of attacking DNA and oxidizing guanine bases resulting in DNA strand breaks.

Materials and Methods

Cell culture- BE-WT and BE-NQ human colon carcinoma cells (gift from David Ross, University of Colorado Health Sciences Center, Denver, CO) were grown in Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 200 mM L-glutamine and 100 mM sodium pyruvate. All media were also supplemented with penicillin at 10,000 U/mL and streptomycin at 10,000 µg/mL. Cell culture medium and supplements were obtained from Mediatech, Inc., Manassas, VA. The cells were incubated at 37°C under a humidified atmosphere containing 5% CO_2.

Growth Inhibition assay- Growth inhibition was determined using the MTT colorimetric assay. Cells were plated into triplicate 96-well plates at a density of 10,000 cells/mL and allowed to attach overnight (16h). Quinolinequinone solutions were applied in medium for 24 hours. Quinolinequinone solutions were removed and replaced with fresh medium and 96-well plates were incubated for 1-2 days. MTT (50 µL) was added and the cells were incubated for another 4 hours. 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 560 nm. IC_{50} values (concentration at which cell survival equals 50% of control) were determined from semilog plots of percent of control *vs.* concentration. Selectivity ratios (SR) were defined as the IC_{50} value for the BE-WT cell line divided by the IC_{50} value for the BE-NQ cell line.

NQO1 activity assay- NQO1 activity in BE-WT and BE-NQ cells was determined spectrophotometrically by monitoring reduction of the standard electron acceptor, 2,6-dichlorophenol-indophenol (DCPIP). Cells were harvested in 25 mM Tris-HCl (pH 7.4) with 250 mM sucrose then sonicated on ice. Reactions contained 200 µM NADH, 40 µM DCPIP, +/- 20 µM dicoumarol and 5-50 µL sonicated cells in 25 mM Tris-HCl (pH 7.4) with 0.7 mg/mL bovine serum albumin and 5 µM FAD. Reactions were followed for 30 s at 600 nm (ε=2.1 x 10^4 M^{-1}). Protein concentration was determined using the BCA protein assay (Pierce, Rockford, IL). Reduction rates are expressed as nmol DCPIP reduced/min/mg total protein after subtracting out rates in the presence of the NQO1 inhibitor, dicumarol. NQO1 activity in the BE-WT cells was found to be 8.6 nmol DCPIP reduced per min per mg total protein compared with 337 nmol DCPIP reduced per min per mg total protein for the NQO1-transfected BE-NQ cells.

Conclusions

- There was a linear relationship between the selectivity ratios of the IC50 values of the MTT assays and the reduction rates of the spectrophotometric assay although it was not statistically significant. (r^2 =0.5088, *P*=0.0719, Table 1 and Figure 1)
- Of the 7 quinolinequinones examined for cytotoxicity, the best substrates for NQO1 showed selective toxicity toward BE-NQ cells. (Table 2)
- Two of the three most selective compounds were HP414 and HP436. This is possibly due to their pyridine substituents that act as part of a molecular cavity with potential metal binding sites. (Figures 2 and 3)
- Further study examining quinolinequinone compounds with amino substituents on the group at the R^2 position would clarify the possible necessity of a stronger metal binding site for increased selective toxicity.

Acknowledgements

This study was supported by the National Institute of Health Science Grant R25ES16247.