Abstract

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 wherein drugs that are bioreductively activated in cancer cells are transformed into highly reactive intermediates capable of attacking DNA and oxidizing guanine bases resulting in the formation of strand breaks. Redox cycling-mediated oxidative stress, depletion of attacking DNA and oxidizing guanine bases resulting in the formation of strand breaks. The NQO1-mediated redox cycling of quinones can produce highly related to the bioreduction process for activation of the quinones. The candidate for enzyme directed bioreductive drug development chemotherapeutic compounds. Marked elevations in NQO1 activity have been observed in some tumor cell lines and may be selectively toxic to those tumors. Some of these compounds have been shown to be selectively toxic to cancer cells in culture. For example, HP414 and HP436. These two compounds contain pyridine substituents that selectively interact with molecular targets unique to tumor cells.

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Table 1. Metabolism of quinolinoquinone compounds by recombinant human NQO1 monitored by spectrophotometric cytochrome c assay (indicating initial reduction rate) and HPCL and calcium cyanide (indicating extended reduction rate). Reduction rates measured in amol/min/mg protein.

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

Figure 1. Correlation of the reduction rates obtained from the spectrophotometric assay and selectivity ratios obtained by dividing the IC50 values of BE-WT (NQO1-expressing) by the IC50 values of BE-NQ (NQO1-rich) cells. Linear regression data include an R2 value of 0.8407 and a p value of 0.019. IC50 values obtained by the MTT assay.

Figure 2. Molecular structure of compound HP436. Metal binding site located in the molecular cavity formed by the quinolinoquinone and pyridine substitutent. Both compounds HP414 and HP436 include pyridine substitutents. In vitro, HP436 showed more selective toxicity toward BE-NQ cell lines.

Figure 3. Cytotoxic mechanism of quinones. The hydroquinone produced after the two-electron reduction causes oxidative stress by attacking DNA and oxidizing guanine bases resulting in DNA strand breaks. DNA strand breaks were measured using the MTT assay. 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). Quinolinoquinone 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 550 nm (wavelength at which cell survival equals 50% of control) were determined from semilog plots of percent of control vs. concentration. Selectivity ratios (SR) were calculated as IC50(BE-WT)/IC50(BE-NQ) for the BE-WT cell line divided by the IC50 value for the BE-NQ cell line.

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Conclusion

There was a linear relationship between the selectivity ratios of the MTT assays and the IC50 values of the spectrophotometric assay although it was not statistically significant. (r2=0.5088, P=0.0719, Table 1 and Figure 1) Of the 7 quinolinoquinones examined for cytotoxicity, the best substrates for NQO1 showed selective toxicity toward BE-NQ cells. 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. Further study examining quinolinoquinone compounds with amino substituents on the group at the R2 position would clarify the possible necessity of a stronger metal binding site for increased selective toxicity.

Acknowledgements

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