Elucidation of Thioredoxin as a Molecular Target for Antitumor Quinols

Tracey D. Bradshaw,1 Charles S. Matthews,1 Jennifer Cookson,1 Eng-Hui Chew,1 Manish Shah,1 Kevin Bailey,1 Anne Monks,2 Erik Harris,3 Andrew D. Westwell,1 Geoffrey Wells,1 Charles A. Laughton,1 and Malcolm F.G. Stevens1

Abstract

Heteroaromatic quinols 4-(benzothiazol-2-yl)-4-hydroxycyclohexa-2,5-dienone (1) and 4-(1-benzenesulfonyl-1H-indol-2-yl)-4-hydroxycyclohexa-2,5-dienone (2) exhibit potent and selective antitumor activity against colon, renal, and breast carcinoma cell lines in vitro (GI50 < 500 nmol/L). In vitro growth inhibition of renal, colon, and breast xenografts has been observed. Profound G2-M cell cycle block accompanied down-regulation of cdk1 gene transcription was corroborated by decreased CDK1 protein expression following treatment of HCT 116 cells with growth inhibitory concentrations of 1 or 2. The chemical structure of the quinol pharmacophore 4-(hydroxycyclohexa-2,5-dienone) suggested that these novel agents would readily react with nucleophiles in a similar manner and suggested that thioredoxin/thioredoxin reductase signal transduction could be a putative target. Molecular modeling predicted covalent irreversible binding between quinol analogues and cysteine residues 32 and 35 of thioredoxin, thereby inhibiting enzyme activity. Binding has been confirmed, via mass spectrometry, between reduced human thioredoxin and 1. Microarray analyses of untreated HCT 116 cells and those exposed to either 1 (1 μmol/L) or 2 (500 nmol/L and 1 μmol/L) determined that of ≥10,000 cancer-related genes, expression of thioredoxin reductase was up-regulated 53-fold. Furthermore, quinols 1 and 2 inhibited insulin reduction, catalyzed by thioredoxin/thioredoxin reductase signaling in a dose-dependent manner (IC50 < 6 μmol/L). Results are consistent with involvement of action of novel antitumor quinols with involving inhibition of the small redox protein thioredoxin. (Cancer Res 2005; 65(9): 3911-9)

Introduction

The discovery of structurally novel experimental antitumor agents with potent and selective activity against germane molecular targets in intractable malignancies represents a challenging endeavor. In recent years, we have been harnessing the power of hypervalent iodine oxidation chemistry to generate structural novelty and diversity by oxidation of biologically relevant phenols. Our studies have led to the synthesis and antitumor evaluation of heteroaromatic-substituted hydroxycyclohexadienones (quinols) such as 4-(benzothiazol-2-yl)-4-hydroxycyclohexa-2,5-dienone (1; Fig. 1; ref. 1). Further chemical syntheses and structure activity screening uncovered a series of (arylsulfonyl)indole-substituted quinols [e.g., 4-(1-benzenesulfonyl-1H-indol-2-yl)-4-hydroxycyclohexa-2,5-dienone (2; Fig. 1) that possess significantly greater growth inhibitory properties in vitro (2). Molecules in both series displayed a highly unusual pattern of selectivity in the National Cancer Institute (NCI) Developmental Therapeutics Program in vitro screen of 60 human-derived cancer cell lines; cytotoxicity was clustered within colon, renal, and certain breast cell lines only (Fig. 2A). Evidence of antitumor activity against renal, colon, and breast xenograft models in vivo has also been obtained (1, 2).

In the context of cancer drug development, the tissue origin of drug-sensitive cell lines is pertinent. Mammary carcinoma is the most common cancer in women, accounting for almost one third of all female cancer cases. The lifetime risk for breast cancer in women is one in nine. Although 5-year survival exceeds 70%, 17% cancer deaths among females are from breast cancer. Worldwide, new cases of colorectal carcinoma exceed one million annually, the second highest incidence after lung cancer; large bowel cancer mortality represents 10% of all cancer deaths. Renal cancer accounts for 2% of all new cancer cases and 2% of cancer deaths; 60% of patients succumbing to colorectal or renal carcinoma will not be alive 5 years after diagnosis. It is clear therefore, that novel treatment strategies for these intractable solid cancers are urgently required.

In our laboratory, two lead molecules have emerged (1 and 2) from the heteroaromatic cyclohexadienones (quinols) program, which elicit potent antitumor activity against HCT 116 and HT29 colon-derived, and MCF-7 and MDA 468 breast-derived human carcinoma cell lines. However, when the intriguing profile of tumor cell growth inhibition and cytotoxicity was initially uncovered, a molecular target was elusive: these agents exacted antitumor activity via unknown mechanism(s) of action.

In this article, we describe the multidisciplinary approach undertaken to elucidate mechanisms involved in the antitumor activity of novel quinols, exposing thioredoxin as a molecular target. The implications of these findings are discussed.

Note: This article represents part 3 in the series "Quinols as novel therapeutic agents." Part 2 is reference 2.

Requests for reprints: Tracey D. Bradshaw, Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, United Kingdom. Phone: 44-115-951-3419; Fax: 44-115-951-3412; E-mail: tracey.bradshaw@nottingham.ac.uk.

©2005 American Association for Cancer Research.
Materials and Methods

The chemical syntheses of 1 and 2 have been described previously (1, 2). Quinol stocks (10 mmol/L) were prepared in DMSO, and stored, protected from light at 4°C for 4 to 6 weeks.

Mammary carcinoma (estrogen receptor–positive (ER+) MCF-7 and estrogen receptor–negative (ER–) MDA-MB-468) and colon carcinoma (HCT 116 and HT29) cell lines were subcultivated twice weekly in RPMI-1640 supplemented with 10% fetal bovine serum and incubated at 37°C in an atmosphere of 95% air, 5% carbon dioxide. To minimize phenotypic drift, untreated samples were modified in the same manner but with Cy5 dye bound to cDNA (reverse dye incorporation). Equal amounts of the two samples were hybridized against five cDNA arrays (Advanced Technology Center, Center for Cancer Research, NC). The ability of 2 (0.5 and 1 μmol/L) to alter gene expression in HCT 116 colon carcinoma cells was determined after 24 hours of exposure. RNA was isolated as described and gene expression evaluated by competitive hybridization of control versus drug-treated cDNA samples as described above, on replicate microarrays containing 20,000 oligonucleotides (Advanced Technology Center, Center for Cancer Research, NC). All data was analyzed through the Computer Information Technology Center’s mAdb web site.4 Gene lists generated were subjected to DAVID and EASE bioinformatic tools developed by the Laboratory of Immunoopathogenesis and Bioinformatics at SAIC, Frederick for the National Institute of Allergy and Infectious Diseases of the NIH. EASE determined GO functional classifications where the abundance of genes in the selected set (up-regulated or down-regulated) was significantly higher (Bonferroni corrected P < 0.01) than that in the Locus Link database, suggesting functional classifications of identified genes, which did not represent random selection.

Western Blot

Whole cell lysates were prepared for examination of protein expression from untreated cultures and following exposure of cells to compounds 1 and 2. Following protein determination (n = 3; ref. 5) and addition of sample buffer, samples were heated to 95°C for 5 minutes and solubilized proteins (50 μg) were separated by SDS-PAGE (10%). Proteins were electrophoresed to polyvinylidene difluoride membranes and probed using an anti-CDK-1 primary antibody (Oncogene Research Products, San Diego, CA) and a secondary antibody conjugated to horseradish peroxidase (Pierce, Rockford, IL). Membranes were then treated with SuperSignal West Pico enzyme chemiluminescence substrate (Pierce) before exposure to X-ray film (Fuji, Tokyo, Japan) for 5 minutes. Membranes were stripped using stripping buffer [0.15 mol/L glycine and 0.1% SDS (pH 2.5)], washed, and blocked before being reprobed with antibodies against thioredoxin (Becton Dickinson, Franklin Lakes, NJ), thioredoxin reductase (TR; Lab Frontier, Seoul, Korea) and actin (Oncogene Research Products) using a similar detection system. Actin levels were used to verify protein loading. Three separate sets of samples were analyzed for all four proteins. Molecular weight markers were included in all blots to confirm detection of proteins of the correct molecular weight.

Mass Spectrometry

Human thioredoxin protein (Inco, Stockholm, Sweden) was dissolved (1 mg/mL, −86 μmol/L) in 100 mmol/L ammonium bicarbonate (pH 8.1)/1 mmol/L tris(2-carboxyethyl)phosphine (TCEP). Compound 1, final

4 http://apps1.niaid.nih.gov/david/
Figure 2. A, activity of compounds 1 and 2 in the NCI cancer cell line panel. Mean LC50 values, following analysis of cytotoxicity using the sulforhodamine B assay after 48 hours of drug exposure. B, effect of compounds 1 and 2 on growth of human-derived MCF-7 breast and HCT 116 colon carcinoma cells. Cells were exposed to quinols for 72 hours before growth and cytotoxicity was analyzed by MTT assay. Points, means of eight readings; bars, SD < 5%. Experiments done on ≥3 separate occasions.
Figure 3. Cell cycle analyses of HCT 116 cell populations after 16 hours (i, iv, and vii), 24 hours (ii, v, and viii), and 48 hours (iii, vi, and ix) of exposure to DMSO vehicle alone (i, ii, and iii), 1 μM/L 1 (iv, v, and vi), and 500 μM/L 2 (vii, viii, and ix); 10,000 events per sample were analyzed. Results

Growth inhibition and cytotoxicity. Cytotoxicity, elicited by quinols 1 and 2 upon human-derived carcinoma cells, is largely clustered within the colon, renal, and breast panels of the NCI 60 cell line screen (Fig. 2A). It may be appreciated from this representation of the data that 2 represents the more potent analogue. In HCT 116, HCT 15 colon, ACHN, CAKI-1 renal, and MCF-7 breast cell lines LC<sub>50</sub> values < 100 nmol/L were obtained. Figure 2B shows results of representative MTT assays done in our laboratory; compound 2 elicits more potent growth inhibition in the two breast-derived and two colon-derived tumor cell lines challenged with compounds 1 and 2 (72 hours). Gl<sub>50</sub> values <500 nmol/L are achieved.

Cell cycle analyses. MCF-7, MDA 468, HCT 116, and HT29 cells, challenged with growth inhibitory concentrations of 1 and 2, show immediate onset of profound G<sub>2</sub>-M cell cycle block, at the expense of G1 and initially, S-phase cells. Representative cell cycle perturbations are illustrated in Fig. 3, following treatment of HCT 116 cells with 1 μM/L 1 and 500 μM/L 2. As treatment times extended beyond 16 hours, numbers of cells within S phase began to increase once more, and a pre-G1 apoptotic peak became evident; however, no significant release of cells blocked within the G<sub>2</sub>-M cell cycle phases was evident.

Insulin Reduction Assay

Microtiter plate colorimetric assays, based on the increase in absorbance at 405 nm, which occurs when dithionitrobenzoic acid (DTNB) is reduced by the enzyme-mediated transfer of reducing equivalents from NADPH, were done for TR/thioredoxin–dependent insulin reducing activity (6). TR/thioredoxin–dependent insulin reducing activity was measured in incubates (final volume, 100 μL) containing 100 mM/L HEPES buffer (pH 7.2), 5 mM/L EDTA (HE buffer), 1 mM/L NADPH, 1 μM/L TR, 0.8 μM/L thioredoxin, and 2.5 mg/mL bovine insulin. Incubations were for 30 minutes at 37°C in flat-bottomed 96-well microtiter plates. The reaction was stopped by the addition of 125 μL of 6 mol/L guanidine-HCl, 50 mM/L Tris (pH 8.0), and 10 mM/L DTNB and absorbances were measured at 405 nm. Appropriate control samples were included in the assay in which TR, thioredoxin, or insulin were omitted from the incubation mixtures. Insulin reduction assays were done in which all reagents were introduced simultaneously. In addition, to elucidate further the inhibitory nature of TR/thioredoxin signal transduction by 1 and 2, reagents excluding insulin were incubated (30 minutes, 37°C) before introduction of insulin. There followed a further 30 minutes incubation at 37°C before the reaction was stopped as described above and absorbance read at 405 nm.

Figure 4. Gene expression changes in RNA isolated from HCT 116 cells following 24 hours of exposure to 1 or 10 μM/L 1 (A) and 500 nmol/L or 1 μM/L 2 (B). A, RNA samples were hybridized against arrays containing 10,000 elements. B, gene expression was evaluated by competitive hybridization of control versus drug-treated cDNA samples on microarrays containing 20,000 oligonucleolets. Genes: TXNRD1, thioredoxin reductase 1; PRKACB, camp-dependent protein kinase; ART3, ADP-ribosyltransferase 3; ZNF177, zinc finger protein 177; ASNS, asparagine synthetase; BTEB1, basic transcription element BP 1; EST, ESTs like ferritin light chain; HMOX1, heme oxygenase (decycling) 1; GDP19, growth differentiation factor 15; CLU, clusterin; C20orf139, chromosome 20 ORF 139; SQSTM1, sequestosome; IL7, interleukin 7; UPP1, uridine phosphorylase 1; C20orf7, chromosome 20 ORF 7.
**Gene array.** Figure 4A summarizes the most prominent genes demonstrating altered expression in HCT 116 cells after treatment with 1 μmol/L I, compared with the same genes after treatment of cells with 10 μmol/L I. Criteria for gene selection required ≥4-fold increase or decrease in gene expression following drug treatment in more than two of the replicate assays. Intriguingly, the only gene whose transcription was induced by 1 μmol/L I and met these criteria was TR. All other selected genes whose transcription was altered following exposure of cells to 1 μmol/L I were down-regulated, although after 10 μmol/L treatment, the zinc finger protein 177 (ZNF177) and the basic transcription element binding protein 1 (BTER) genes showed increased expression. As both these genes are potential transcription regulators, the higher concentration may regulate gene transcription by additional mechanisms. This hypothesis is supported by the observation that following treatment of HCT 116 cells with 10 μmol/L I, and using the same selection criteria, transcription of 32 genes was up regulated and 11 genes down-regulated (data not shown). Among the former, TR was modestly induced (2- to 3-fold; Fig. 4A). The most highly induced genes encoded heat shock proteins, and transcription factors that tended to be stress response genes. Furthermore, transcription of GADD45, the DNA damage response gene was enhanced, as was Il-8, another common stress response gene. The most highly down-regulated gene was that encoding CDK-1, the kinase responsible for cycling of cells to quinol analogues, concordant with the observed cell cycle block and reduced cdk1 gene transcription following treatment of HCT 116 cells with 1 μmol/L I. In contrast, compounds I and 2 evoked increased expression of TR protein, in a dose-dependent manner, again corroborating microarray data. There was no significant alteration in levels of thioredoxin detected in lysates of cells treated with either analogue. Protein content introduced into gel wells remained constant (50 μg) a fact confirmed by detection of actin in each lysate sample.

**Binding of I to thioredoxin.** Incubation of human thioredoxin alone with reductant (TCEP) and DMSO (vehicle control), revealed the predominant peak in the mass spectrum corresponding to reduced thioredoxin (mass = 11,606; Fig. 6A). Following incubation with 1 (1 mmol/L final concentration), significantly altered spectra were detected. A mass peak of 12,336 corresponded to thioredoxin plus three molecules of I. Further mass peaks of 12,586 and 12,821 were consistent with thioredoxin plus four and five bound molecules of I, respectively (Fig. 6B). The latter two masses differ by ~0.03% and 0.04% from the calculated expected mass, thought to be caused by reduced quality of spectra upon incubation with drug, as drug-bound protein did not ionize or “fly” as well in the mass spectrometer as the control incubated thioredoxin. A peak corresponding to nonmodified thioredoxin was not present after drug incubation, suggesting that all the protein had bound drug.

According to our hypothesis, quinol molecules bind Cys residue(s) of thioredoxin. From the fragmentation ion data, sequence identification was possible for the majority of the component amino acids and the additional mass of I was indeed shown present only at Cys residues (Fig. 7). The expected peptides resulting from a theoretical tryptic digestion of human thioredoxin are shown in Table 1.

For control incubations (thioredoxin plus reductant plus DMSO vehicle), the predicted peptide fragment of weight 1,624 corresponding to residues 22 to 36 of the protein (inclusive) was detected as a 2+ ion of mass/charge 812 (Fig. 7A). This peptide fragment incorporates the redox active site region of thioredoxin (residues 32-35). In the digested drug-incubated sample, this mass was not detected but fragments of 1,867 mass units (residues 22-36 plus one molecule of I) and 2,110 mass units (+2 molecules of I) were detected, as 2+ ions of mass/charge 934 and 1,055, respectively (Fig. 7B and C).

A 2+ ion of mass/charge 732, which would correspond to the expected peptide fragment constituted by residues 37-48 (expected mass 1463), a region incorporating no Cys residues, was detected in both the control and drug-incubated protein samples. The peptide corresponding to residues 49 to 72 (incorporating 2 Cys residues), expected mass 2,719, was also detected as a 3+-ion of mass/charge 907. The drug-incubated protein did not confer this ion but a 3+ ion of mass/charge 988, which would correspond to the peptide fragment of residues 48 to 71 plus one (only) molecule of I.

**Protein expression.** Representative Western blots, following detection of thioredoxin, CDK1, TR, and loading control actin in lysates of HCT 116 cells are shown in Fig. 5. Cells were exposed either to 1, 2 (100, 500 nmol/L, and 1 μmol/L), or DMSO vehicle for 24 hours before preparation of cell lysates. Dose-dependent down-regulation of CDK1 protein was detected following exposure of cells to both quinol analogues, concordant with the observed cell cycle block and reduced cdk1 gene transcription following treatment of HCT 116 cells with 1 μmol/L I. In contrast, compounds 1 and 2 evoked increased expression of TR protein, in a dose-dependent manner, again corroborating microarray data. There was no significant alteration in levels of thioredoxin detected in lysates of cells treated with either analogue. Protein

![Figure 5](image-url)
(expected calculated mass of 2,962). The peptide corresponding to residues 73 to 81 (incorporating one Cys residue), expected mass of 1,148, was detected as a 2+ ion of mass/charge 574 in the control incubation and not in the drug-incubated sample. For the latter, a 2+ ion of mass/charge 696, which would correspond to the peptide fragment comprising residues 73 to 81 plus one bound molecule of 1 (expected calculated mass of 1,391) was detected (results not shown).

**Insulin reduction.** The ability of quinol analogues to inhibit TR/thioredoxin–mediated signal transduction was examined exploiting the ability of this pathway to reduce insulin. When reaction mixtures including all reagents were incubated for 30 minutes at 37°C, analogues 1 and 2 inhibited insulin reduction dose-dependently, yielding IC50 values of 28 and 37.14 μmol/L, respectively (Fig. 8). Extended incubation times (60 minutes) failed to have any notable effect on insulin reduction. Interestingly, when reaction mixtures, excluding insulin were incubated for 30 minutes (37°C), then a further 30 minutes upon addition of insulin, insulin reduction by TR/thioredoxin signal transduction in the presence of these molecules was significantly decreased.
(IC_{50} values 2.68 and 5.95 μmol/L, respectively, were obtained for 1 and 2; Ps < 0.001). These comparative observations concur exactly with previous experiments using Escherichia coli thioredoxin and TR as signal transducers for the reduction of insulin (results not shown).

Discussion
To begin to probe the mechanism(s) of action of this class of experimental antitumor agents, COMPARE analysis (7) using 1 as seed compound was done. Such data mining revealed a number of natural products [e.g., 22-hydroxytingenone (NSC 684506), heliangolide (NSC 335753), and arnebin (NSC 140377)] together with small synthetic molecules, sharing similar profiles of antitumor activity and characterized chemically as “double Michael acceptors.” A Michael acceptor is defined as a molecule containing an α,β-unsaturated carbonyl function able to form covalent bonds with sulfur nucleophiles (thiols) at the β-carbon position. Pearson correlation coefficients (>0.7), suggested mechanistic similarity between 1 and these disparate structures that elicited toxicity in colon, renal, and certain breast cell lines (6). Thus, a platform for interrogation of the database for molecular targets expressed in different cell types was provided. Of interest was TR/thioredoxin signal transduction.

Figure 7. Peptide sequencing reports for human thioredoxin following tryptic digest. Peptides comprising amino acid residues 21 to 35. A, DMSO control incubated protein. B and C, drug-incubated (1 mmol/L) protein. Incubations (15 minutes) preceded digestion at 37 °C overnight using a 1:6 ratio of trypsin to thioredoxin in ammonium bicarbonate/TCEP. Digestions were stopped by the addition of formic acid. Analyses by MS revealed resultant peptide fragments and subsequent MS/MS enabled sequencing. Observed weights of fragments and cysteine residues are circled: additional weight of 1.
electrophilic human thioredoxin with the interatomic distance between the comparing the Cys 32-Cys 35 sulfur interatomic distance in residue to form an irreversible complex (8). Structural studies monosulfur adduct, followed by addition of a second Cys thiol thiol residue of Cys (32 or 35) of thioredoxin, to give a reversible catalyzed reduction of insulin (IC50 <6
Indeed, powerful dose-dependent inhibition of TR/thioredoxin-consequence of which would be inhibition of signal transduction. Thioredoxin may act as a growth factor and promote aggressive tumor growth, angiogenesis, inhibit apoptosis, and augment resistance within tumors to chemotherapeutic agents such as cisplatin. Thioredoxin regulates HIF-1α protein levels under normoxia and hypoxia. Thioredoxin transfection has been shown to elicit significantly elevated hypoxia-induced HIF-1 transactivation activity resulting in a significant increase in the protein products of hypoxia-responsive genes such as vascular endothelial growth factor (VEGF). Indeed, VEGF production by tumor cells was reduced by 1, and colorectal carcinoma cell lines showed enhanced sensitivity to 1 under hypoxic conditions (16).

Gene array analyses of HCT 116 colon carcinoma cells showed that of ≥10,000 cancer-related genes, expression of only one, TR, was enhanced >4-fold following exposure of cells to 1 (1 μmol/L) for 24 hours. Following treatment of HCT 116 cells with 2, the most significantly induced gene (>3-fold increase in expression) was again TR (Fig. 4). Such up-regulation of TR suggests an attempt by the cell to compensate for the enhanced oxidative stress inhibition of thioredoxin would trigger, and provides further corroboration of a mechanism of action that includes inhibition of thioredoxin. Indeed, transcription of HO-1, a protein highly induced in response to oxidative stress (17), was increased dose-dependently by 2.

Whereas our evidence supports molecular interaction between heteroaromatic quinols and thioredoxin, we acknowledge the probable involvement of other targets and mechanisms in antitumor activity. For example, protein-disulfide isomerases (PDI), which offer protection against apoptosis and provide chaperone activity, show sequence and structural homology to thioredoxin, discussed herein, together with mitochondrial thioredoxin-2 serve to protect the cell against oxidative stress and both are essential for embryonic development (10). Thioredoxin, an important mediator of redox regulation also modulates the actions of crucial cellular enzymes and transcription factors (11, 12). Thioredoxin interacts with redox factor-1 (Ref-1), modifying the binding activity of activator protein-1 (AP-1). Thioredoxin activates NF-κB evoking cellular responses to oxidative stress, tumorigenesis, and apoptosis. Reduced thioredoxin prevents apoptosis by complexing apotosis signal regulating kinase-1 (ASK-1). When thioredoxin is oxidized by reactive oxygen species, binding between ASK-1 and thioredoxin dissociates, and apoptosis signal transduction is activated (13). Elevated thioredoxin and TR, found in many human tumors including lung, colon, hepatoma, and pancreas (14, 15), are associated with poor prognosis and decreased patient survival. Thioredoxin, a 12-kDa protein, is a potent intracellular disulfide reductase possessing key roles in the regulation of biological functions such as cellular proliferation (deoxyribonucleotide biosynthesis), growth control, and apoptosis. Cytoplasmic/nuclear thioredoxin

<table>
<thead>
<tr>
<th>Amino acid residues comprising peptides</th>
<th>Expected weight [M + H]</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>245.18</td>
<td>MVK</td>
</tr>
<tr>
<td>4-8</td>
<td>603.33</td>
<td>QIESK</td>
</tr>
<tr>
<td>9-21</td>
<td>1,336.64</td>
<td>TAFAQELDAAGDK</td>
</tr>
<tr>
<td>22-36</td>
<td>1,624.79</td>
<td>LVVVDSATWCPCK</td>
</tr>
<tr>
<td>37-48</td>
<td>1,463.77</td>
<td>MIKPPFHSLSEK</td>
</tr>
<tr>
<td>49-72</td>
<td>2,719.21</td>
<td>YSNVIFLEVDQDVASECEVK</td>
</tr>
<tr>
<td>73-81</td>
<td>1,148.53</td>
<td>CMPTFFQFK</td>
</tr>
<tr>
<td>82-82</td>
<td>147.11</td>
<td>K</td>
</tr>
<tr>
<td>83-85</td>
<td>392.19</td>
<td>QVK</td>
</tr>
<tr>
<td>86-94</td>
<td>908.45</td>
<td>VGEFGANK</td>
</tr>
<tr>
<td>95-96</td>
<td>276.16</td>
<td>EK</td>
</tr>
<tr>
<td>97-105</td>
<td>1,001.55</td>
<td>EATINELV</td>
</tr>
</tbody>
</table>

NOTE: Cys residues in bold font.

Figure 8. Inhibition of thioredoxin/TR–catalyzed reduction of insulin by (A) 1 and (B) 2.
Cys residues may be susceptible to covalent interaction with quinol analogues. Indeed, experiments investigating interactions between 1 and EndoPDI are under way.5 EndoPDI, present in tumor endothelium, but rarely expressed in normal tissues, is induced by hypoxia, acting as a stress survival factor (18). Growth inhibition of proliferating human vein endothelial cells by 1 has been shown (16), tube formation was aborted, suggesting inhibition of endothelial differentiation, an observation also consistent with the antiangiogenic activity of 1.

Microarrays showed down-regulation of cdk-1 gene expression, translating to dose-dependent decreases in CDK-1 protein expression (Fig. 5) in lysates of cells treated with 1 or 2, and accompanied by profound perturbation in cell cycle profiles (Fig. 5). CDK1 is integral to G2-M progression and its expression has been shown to be under the partial control of CDK-1 elements within its promoter (19). When coupled with the ability of thioredoxin to activate AP-1 through direct interaction with DNA repair protein and Ref-1 (20), it could be argued that inhibition of thioredoxin activity may decrease AP-1 transcription of the cdk-1 gene. Transcription of GADD45, whose protein product accumulates following DNA damage and is implicated in cell cycle control and DNA repair in response to genotoxic stress, is up-regulated by 1. GADD45 causes G2-M cell cycle arrest through direct interaction and disruption of CDK-1/cyclin B1 complexes (21). 7-Hydroxystaurosporine (UCN-01), the DNA repair inhibitor, capable of abrogating G2 arrest (22–24) via a mechanism which includes inhibition of CDK-1 phosphorylation, failed to influence G2-M cell cycle blocks induced by 1 and 2. Similarly, cotreatment of cells with UCN-01 and 1 or 2 did not potentiate quinol cytotoxicity (results not shown). Finally, trans- scription of asparagine synthetase was down-regulated by 1 and 10 μmol/L. 1, raising the possibility that l-asparagine amidohydrolase (asparaginase) and 1 may represent an effective experimental treatment combination.

We believe that modulation of thioredoxin represents a valid therapeutic goal; indeed, one other inhibitor of thioredoxin is currently undergoing clinical evaluation. The disulfide PX12, irreversibly thioalkylates the noncatalytic site Cys73 of thioredoxin, thereby disabling its ability to be reduced by TR (25). Thus, compounds 1 and 2 represent a distinct mechanistic class of thioredoxin inhibitor; they are synthetically accessible, and show selectivity and potency against breast, colon, and renal cell lines in vitro and in vivo. Importantly, we have shown that this class of molecular targets thioredoxin, a protein possessing key roles in the etiology of malignant disease.

Acknowledgments

Received 11/18/2004; revised 2/3/2005; accepted 2/16/2005.


The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank SCI for collaborations and for critical appraisal of the article, Stewart Martin, and Abhik Mukherjee.

References

Elucidation of Thioredoxin as a Molecular Target for Antitumor Quinols

Tracey D. Bradshaw, Charles S. Matthews, Jennifer Cookson, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/9/3911

Cited articles
This article cites 25 articles, 8 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/9/3911.full.html#ref-list-1

Citing articles
This article has been cited by 11 HighWire-hosted articles. Access the articles at:
/content/65/9/3911.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.