



Enzymology of bioreductive drug activation

D Ross, HD Beall, D Siegel, RD Traver and DL Gustafson

School of Pharmacy and Cancer Center, University of Colorado Health Sciences Center, Box C-238, 4200 E Ninth Avenue, Denver, CO 80262, USA.

Keywords: P450 reductase; NQO1; DT-diaphorase; xanthine oxidase; mitomycin C; EO9; tirapazamine

Background and current status

The hypoxic fraction of solid tumours is resistant to ionising radiation and some chemotherapeutic agents (Sartorelli *et al.*, 1994). Drugs requiring bioreductive activation have been developed to exploit the oxygen deficiency in these cells on the premise that hypoxic cells should show a greater propensity for reductive metabolism than well-oxygenated cells (Lin *et al.*, 1972). Hypoxia should also limit interactions of the reduced drug with molecular oxygen thereby maintaining the drug in the reduced, cytotoxic form. Much of the recent work in this area has focused on specific enzymes involved in bioreductive drug activation, and this has been more recently described as enzyme-directed bioreductive drug development (Workman and Walton, 1990). Appropriate characteristics of enzyme-directed bioreductive agents have been discussed (Workman, 1994) and these are summarised in Table I. They include knowledge of the role of a particular reductase in bioactivation of a drug and whether there is elevated expression of that reductase in a particular tumour type (Table I). Using this approach a bioreductive drug could be targeted against a particular tumour type. Additional steps for bioreductive drug development such as activity in xenografts and in clinical trials, individual patient profiling for activities of various reductases and the generation of a computerised database are also shown in Table I. A computerised database has proved particularly important in elucidating the role of various reductases in the toxicity of drugs to cell lines in the NCI panel (Paull *et al.*, 1994) and will be discussed below. The enzymology of bioreductive drug activation has posed some problems owing to its complexity, but it has also offered some important opportunities for drug development. In this review, the enzymes which activate the most commonly studied bioreductive drugs and some of the problems associated with bioreductive drug research will be discussed.

Mitomycin C (Figure 1) is the prototype bioreductive alkylating agent (Sartorelli *et al.*, 1994) and a clinically useful agent in the treatment of solid tumours (Crooke and Bradner, 1976). In fact, mitomycin C is the single most active agent for the treatment of non-small-cell lung cancer (Spain, 1993). The reductive activation of mitomycin C was first described in a classic paper by Iyer and Szybalski (1964). Various bioreductive enzymes (Table II) have been implicated in the bioreductive activation of mitomycin C including NAD (P)H:quinone oxidoreductase, also known as NQO1 or DT-diaphorase (Siegel *et al.*, 1990, 1992; Marshall *et al.*, 1989, 1991a; Begleiter *et al.*, 1988, 1989; Dulhanty and Whitmore, 1991), NADPH:cytochrome P-450 reductase (Bligh *et al.*, 1990; Keyes *et al.*, 1984; Pan *et al.*, 1984; Komiyama *et al.*, 1982; Bachur *et al.*, 1979), NADH:cytochrome b₅ reductase (Hodnick and Sartorelli, 1993), xanthine oxidase (Pan *et al.*, 1984) and xanthine dehydrogenase (Gustafson and Pritsos, 1992a). The

roles of another form of NAD(P)H:quinone oxidoreductase, NQO2 (Jaiswal, 1994), and carbonyl reductase (Forrest *et al.*, 1991) in the metabolism of mitomycin C are unclear. One-electron reduction of mitomycin C can lead to generation of reactive oxygen species whereas either one- or two-electron reduction can produce DNA monoalkylation products or DNA cross-links (reviewed by Sartorelli *et al.*, 1994). It should be emphasised that bioreductive metabolism is only the first step in expression of cytotoxicity, and many other factors such as the behavior of one-electron and two-electron reduced forms under normoxia and hypoxia, the type of cell damage produced (e.g. DNA cross-linking, initiation of apoptosis, etc.) and repair of that damage should be incorporated into any predictive models. This paradigm, although still markedly oversimplified, is shown in Figure 2. Our lack of knowledge of other steps in cellular damage and repair may explain why cells which have markedly increased levels of bioreductive enzymes often show only modest increases in toxicity after treatment with bioreductive agents (Gibson *et al.*, 1992a,b).

The indoloquinone EO9 (Figure 1) is a promising bioreductive drug which is currently in phase I/II evaluation with the EORTC (Plumb *et al.*, 1994). EO9 is a much better substrate for NQO1 than mitomycin C, and the reduced form of EO9 redox cycles more efficiently than the reduced form of mitomycin C (Beall *et al.*, 1995). DNA single-strand breaks have been reported following reduction of EO9 by NQO1 (Walton *et al.*, 1991), and more recently, it was shown that reductive activation of EO9 by NQO1 and xanthine oxidase produced DNA cross-links under hypoxic conditions (Maliepaard *et al.*, 1995). A series of aziridinyl benzoquinones has been studied as potential bioreductive drugs (Gibson *et al.*, 1992a) including diaziquone which has been investigated clinically (Alley *et al.*, 1994). MeDZQ (Figure 1), an analogue of diaziquone, is a better substrate for NQO1 than diaziquone (Beall *et al.*, 1994), and it shows an absolute requirement for reduction to cross-link DNA (Lee *et al.*, 1992). Streptonigrin (Figure 1) is a hydroxyl radical-generating quinone (Cone *et al.*, 1976) that is efficiently activated by NQO1 (Beall *et al.*, 1995).

Other compounds such as the aromatic heterocyclic di-N-oxides are bioactivated by one-electron reduction, and two-electron reduction may represent a detoxification reaction (Walton and Workman, 1993). Bioactivation of tirapazamine

Table I Appropriate characteristics of enzyme-directed bioreductives and steps in drug development

Can the enzyme activate the drug?
Are there increased enzyme activities in tumours?
Is there a relationship between enzyme activity and a biochemical end point of cell damage or cytotoxicity?
Is there a similar relationship in xenografts?
Clinical trials?
Individual patient profiling?
Computerised database?

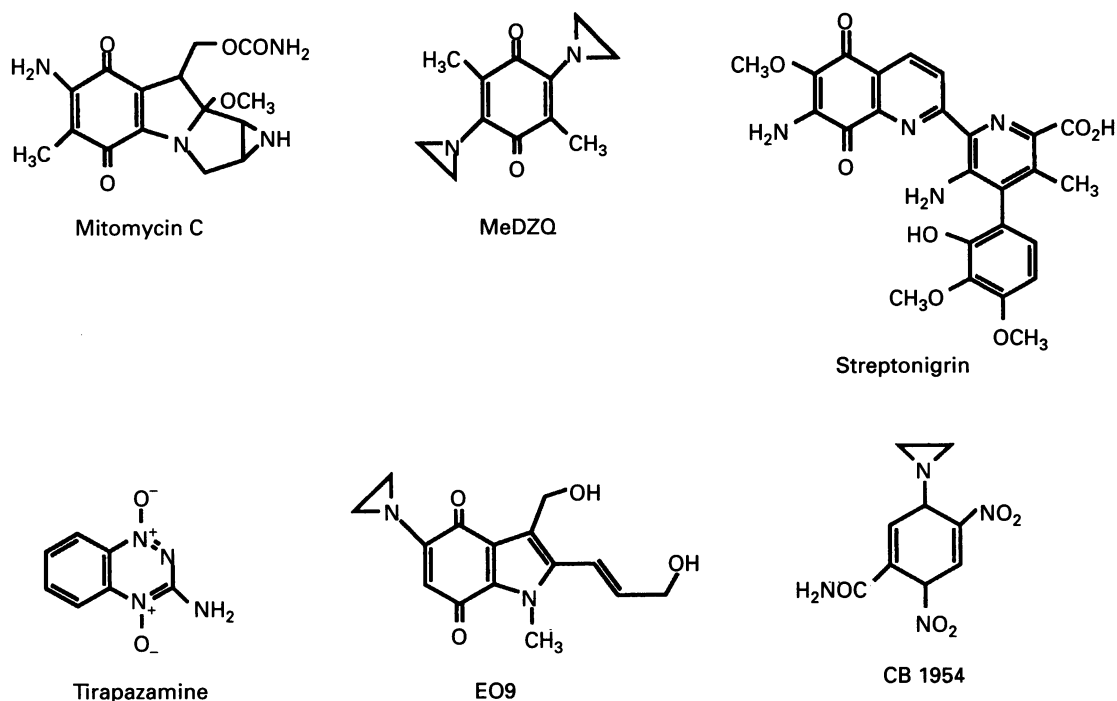


Figure 1 Structures of bioreductive drugs.

(Figure 1), the prototype of this series, can be accomplished with cytochrome P-450 reductase, cytochrome P450 (various isozymes), and xanthine oxidase while NQO1 can detoxify tirapazamine (Patterson *et al.*, 1994). Single- and double-strand breaks in DNA have been reported following activation of tirapazamine by xanthine oxidase under hypoxia (Laderoute *et al.*, 1988). The dinitrophenylaziridines represent another class of bioreductive drugs. CB1954 (Figure 1) is the lead compound in this series in which the 4-nitro substituent is reduced to a 4-hydroxylamino group in a four-electron reduction, although this compound is a much less efficient substrate for NQO1 than mitomycin C (Beall *et al.*, 1994). NQO1 has been implicated in this reaction both in air and under hypoxia whereas cytochrome P-450 reductase and xanthine oxidase, which are one-electron reductases, generate the nitro radical anion which redox cycles under aerobic conditions (Knox *et al.*, 1988). Acetylation of the hydroxylamino group generates a bifunctional alkylating agent, the proposed ultimate DNA cross-linking species (Knox *et al.*, 1991).

NQO1 has generated the most interest of the potential bioactivating enzymes because of its marked overexpression in many tumours and tumour cell lines (Ross *et al.*, 1994; Workman, 1994). Elevations in NQO1 activity or mRNA have been observed in preneoplastic tissues, established tumours or tumour cell lines from human lung, liver, colon, breast and brain (Schlager and Powis, 1990; Malkinson *et al.*, 1992; Cresteil and Jaiswal, 1991; Workman *et al.*, 1991; Berger *et al.*, 1985). Other tumour tissues, including human kidney and stomach, have been found to have decreased

NQO1 activity relative to normal tissue (Schlager and Powis, 1990). The NQO1 activity in human non-small-cell lung tumours and paired normal tissue is shown in Figure 3, illustrating the differences in NQO1 levels between normal and tumour tissue. It should be stressed, however, that although NQO1 is elevated in some tumours such as non-small-cell lung cancer (Malkinson *et al.*, 1992), there is a marked variation in NQO1 activity within a specific tumour type (Figure 4). The variation in NQO1 activity in human tumours is also readily apparent from the data shown in Figure 3.

NQO1 is one of the four diaphorases (diaphorase-4) identified by Edwards *et al.* (1980) corresponding to four gene loci. A diaphorase, or 'coenzyme factor', catalyses the transfer of electrons from reduced pyridine nucleotides to oxidation-reduction indicators (Dewan and Green, 1938; Corran *et al.*, 1939). NQO1 is a two- (Iyanagi and Yamazaki, 1970) or four-electron reductase (Huang *et al.*, 1979) characterised by its unique ability to use both NADH and NADPH as reducing cofactors and its inhibition by dicumarol (Ernster, 1967). A flavoprotein, NQO1 exists as a homodimer with 1 mol of FAD per mol of NQO1 (Lind *et al.*, 1990). Human NQO1 has been cloned and sequenced (Jaiswal *et al.*, 1988), and the crystal structure of the rat liver homologue has recently been reported (Li *et al.*, 1995). Human NQO1 is similar to rat NQO1 with 83% homology for the cDNA and 85% homology for the protein (Jaiswal *et al.*, 1988). Another form of NAD(P)H:quinone oxidoreductase has been isolated, cloned and sequenced and has been designated NQO2 (Jaiswal *et al.*, 1990).

Table II Bioreductive enzymes

NAD(P)H:quinone oxidoreductase1, NQO1
NAD(P)H:quinone oxidoreductase2, NQO2
NADPH:cytochrome P-450 reductase
NADH:cytochrome <i>b</i> ₅ reductase
Xanthine oxidase
Xanthine dehydrogenase
Carbonyl reductase

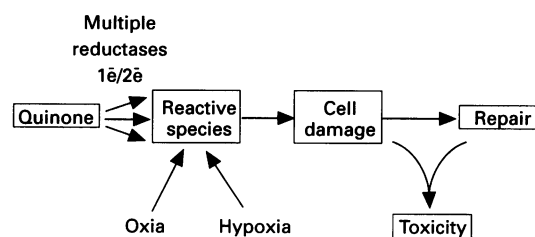


Figure 2 Paradigm for bioreductive activation of quinones.

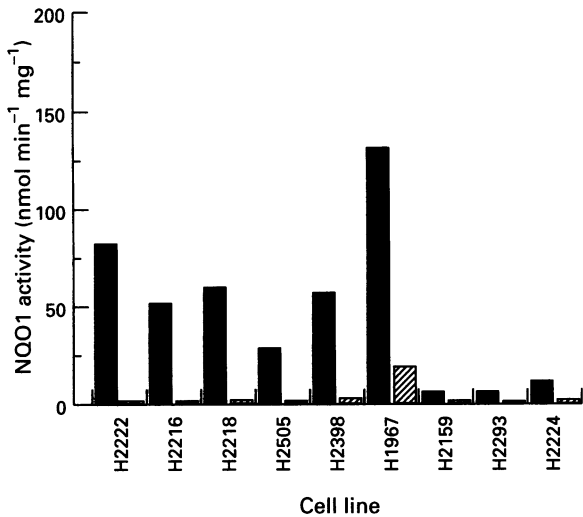


Figure 3 NQO1 activity in non-small-cell lung tumour samples (■) and associated normal tissue (▨). Mean \pm s.e.m. are shown where $n=3$ determinations (Malkinson *et al.*, 1992).

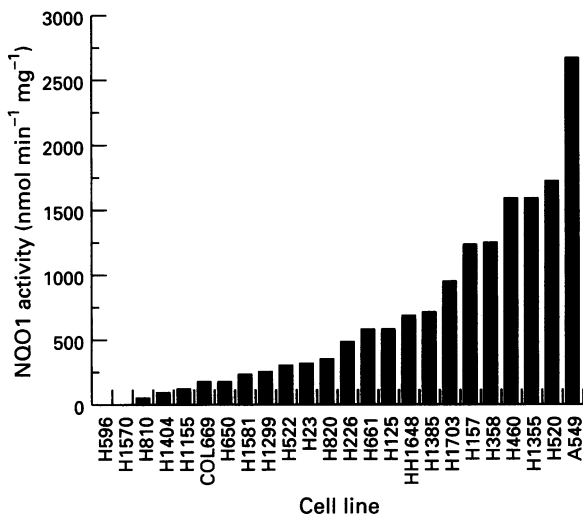


Figure 4 NQO1 activity in non-small-cell lung cancer cell lines (Malkinson *et al.*, 1992).

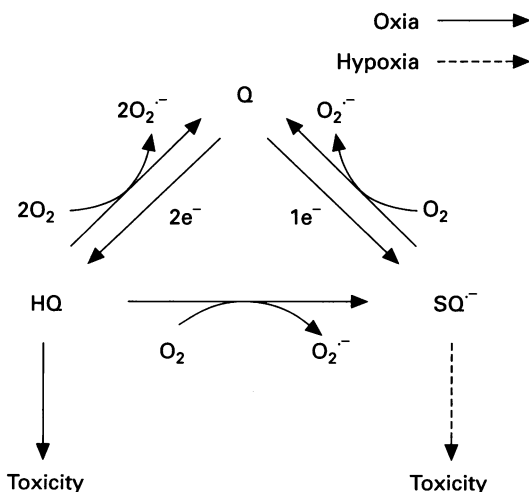


Figure 5 Bioactivation of quinones under aerobic and hypoxic conditions. Q, quinone; HQ, hydroquinone; SQ, semiquinone.

The role of NQO1 in the bioactivation and toxicity of mitomycin C has been controversial. Although metabolism of mitomycin C by NQO1 is pH-dependent owing to pH-dependent inactivation of the enzyme (Siegel *et al.*, 1993), mitomycin C can be bioactivated by NQO1 in the physiological pH range of 7.0–7.4 (Siegel *et al.*, 1990, 1992). These results indicate that NQO1 can bioactivate mitomycin C at physiological pH, but do not imply that NQO1 is the sole reductase catalysing bioactivation of mitomycin C in cellular systems. The role of NQO1 in the toxicity of mitomycin C in tumour cells is controversial. Evidence for an association between NQO1 activity and mitomycin C sensitivity has been reported in both cell lines (Siegel *et al.*, 1990; Begleiter *et al.*, 1989; Dulhanty and Whitmore, 1991; Marshall *et al.*, 1991a), and xenografts (Malkinson *et al.*, 1992), but the role of NQO1 has been questioned because of a lack of correlation between mitomycin C sensitivity and NQO1 activity in a tumour cell line panel (Robertson *et al.*, 1992). More recently, however, we demonstrated a significant correlation between NQO1 activity and mitomycin C sensitivity in human lung and breast cancer cell lines (Beall *et al.*, 1995), and a highly significant correlation ($r=0.424$, $P<0.0005$) was reported in the NCI panel of 69 human tumour cell lines (Fitzsimmons *et al.*, 1996). Of all of these observations the latter provides the strongest support for a potential role of NQO1 in bioactivation of mitomycin C but this evidence is, by nature, indirect. It could also be reasonably argued that, from a toxicological perspective, one might not expect a correlation of NQO1 activity with toxicity, but the situation may be better represented by a threshold model. Correlational studies, however, do not imply causality. The only mechanistic evidence supporting a role for NQO1 in the toxicity of mitomycin C in cells is the data of Hodnick *et al.* (1995) who showed that both mitomycin C and porfirimycin toxicity could be potentiated in CHO cells transfected with NQO1 cDNA. This observation is the first direct demonstration of the role of NQO1 in the bioactivation of mitomycin C in cells. However, in view of the lower catalytic efficiency of human NQO1 as compared with rat NQO1 (see below), these experiments need to be performed using human NQO1.

The importance of NQO1 in EO9 metabolism is somewhat clearer. EO9 can be bioactivated by NQO1 (Walton *et al.*, 1991), and correlations between sensitivity to EO9 and NQO1 levels have been reported in tumour cell line panels (Robertson *et al.*, 1992, 1994; Smitskamp-Wilms *et al.*, 1994). In the NCI panel, EO9 also had a highly significant correlation with NQO1 activity ($r=0.446$, $P<0.0013$; Fitzsimmons *et al.*, 1996). A role for NQO1 in the mechanism of toxicity of both EO9 and mitomycin C has been suggested in a study using NSCLC lines made resistant to mitomycin C which were also cross-resistant to EO9 (Shibata *et al.*, 1995).

The situation becomes much more complex when chemosensitivity studies are conducted under aerobic and hypoxic conditions. Work by Sartorelli's group in the 1980s suggested that NQO1 may bioactivate mitomycin C in well-oxygenated cells, but may protect against mitomycin C-induced cytotoxicity in hypoxic cells (Keyes *et al.*, 1984, 1985, 1989). These studies used the NQO1 inhibitor dicumarol which may have other effects on cellular metabolism as well (reviewed by Ross *et al.*, 1993). In fact, dicumarol was found to increase the hypoxic toxicity of mitomycin C to L1210 murine leukaemia cells which have no measurable NQO1 activity (Keyes *et al.*, 1989). Perhaps most notably, dicumarol has been shown to exert marked stimulatory effects on xanthine dehydrogenase-mediated metabolism of mitomycin C (Gustafson and Pritsos, 1992b) and inhibitory effects on cytochrome *b₅* reductase (Hodnick and Sartorelli, 1993). More recent work has used two human colon carcinoma cell lines; HT-29 with high NQO1 activity and BE with undetectable NQO1 activity due to a polymorphism in the NQO1 gene (Traver *et al.*, 1992). Under aerobic conditions, it was found that mitomycin C was much more toxic to the

high NQO1 HT-29 cells than the NQO1-deficient BE cells. Under hypoxic conditions, sensitivity was essentially unchanged in the HT-29 cells, but was markedly enhanced in the BE cells suggesting the involvement of a one-electron reductase in the enhancement of mitomycin C toxicity in BE cells (Beall *et al.*, 1994). Plumb and Workman (1994) reported similar results for mitomycin C, but the differences in sensitivity were more dramatic with EO9. While sensitivity to EO9 in the HT-29 cells was moderately increased under hypoxia, EO9 toxicity in the BE cells was enhanced by over 1000-fold in hypoxic vs aerobic conditions (Plumb and Workman, 1994). Other reductases in these two cell lines are present at low levels with similar activities (Beall *et al.*, 1994; Plumb and Workman, 1994).

From the data above, it seems clear that the enhanced hypoxic toxicity of quinones is not a function of NQO1-mediated metabolism, but depends on the activity of one-electron reductases. It is not clear, however, whether the one-electron or two-electron reduced forms of quinones or both are responsible for cytotoxicity, and mechanisms may be proposed for either the semiquinone or hydroquinone as the toxic species. The mechanism is complicated not only by interactions of the two reduced species with molecular oxygen, but also by disproportionation and comproportionation reactions between the quinones and their two reduced forms. A possible scheme for bioactivation of quinones is presented in Figure 5. Disproportionation and comproportionation reactions have not been included for simplicity.

A recent study (Plumb *et al.*, 1994) supports the hypothesis that it is the semiquinone that is the proximate toxin. In a panel of cell lines, they demonstrated that the expected correlation between NQO1 activity and EO9 toxicity did exist, but they also showed that there was an inverse correlation under hypoxia, that is, sensitivity decreased with increasing NQO1 activity (Plumb *et al.*, 1994). This suggests that NQO1 protects the cell from EO9 toxicity under hypoxia because autooxidation of the hydroquinone to the more toxic semiquinone is inhibited by the decreased oxygen concentration. The reduced form of EO9 is known to be unstable to oxygen (Beall *et al.*, 1995; J Butler, personal communication). Moreover, in the presence of dicumarol, the inverse correlation observed under hypoxia was lost providing additional evidence for the role of NQO1 in protecting the cells in hypoxic conditions. They also observed that there was a hypoxic/oxic differential for EO9 toxicity in all cell lines with the exception of the A549 lung tumour cell line (highest NQO1 activity) and that the highest differentials were found in those cell lines with the lowest NQO1 activities (Plumb *et al.*, 1994). This could support the premise that the semiquinone is the most toxic species, as diminished oxygen levels would stabilise the semiquinone leading to greater toxicity under hypoxia especially in those cell lines where NQO1 levels are low and the putative protective effects of this enzyme are absent.

The aerobic/hypoxic controversy can also be extended to the one-electron reductase, cytochrome P-450 reductase. Surprisingly, cytochrome P-450 reductase has been implicated in the activation of mitomycin C under aerobic, but not hypoxic conditions (Hoban *et al.*, 1990; Bligh *et al.*, 1990).

Table III Rates of reduction of bioreductive drugs by rat and human recombinant NQO1 (Beall *et al.*, 1995) and percentage efficiency of the human enzyme vs the rat enzyme

Compound	Velocity ^a ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)		Human/rat (%)
	Rat NQO1	Human NQO1	
Streptonigrin	243 ± 42	51 ± 4	21
MeDZQ	104 ± 31	25 ± 4	24
EO9	181 ± 19	7.7 ± 2.0	4
Mitomycin C	0.68 ± 0.02	0.15 ± 0.01	22
CB1954 ^b	0.036 ± 0.003	0.010 ± 0.005	28

^aMean ± s.d. for at least $n = 3$ reactions. ^b100 μM CB1954; all others 50 μM .

However, Belcourt *et al.* (1995) found that CHO cells which were transfected with cytochrome P-450 reductase were more sensitive to mitomycin C and porfiromycin under both aerobic and hypoxic conditions. Sensitivity of the clones to mitomycin C and porfiromycin was greater under hypoxic conditions than in air, and they concluded that cytochrome P-450 reductase contributed to the hypoxic/oxic differential sensitivity of the cells to mitomycin C and porfiromycin (Belcourt *et al.*, 1995).

Search for efficient substrates for bioreductive enzymes

In the search for efficient substrates for the bioreductive enzymes, one approach has been to attempt to correlate reduction potentials to rates of reduction (reviewed by Cadenas, 1995). For the one-electron reductase, xanthine oxidase, Clarke *et al.* (1980, 1982) determined that there was a good correlation between one-electron reduction potential and rates of reduction of nitroimidazoles. A relationship between one-electron reduction potential and rates of reduction by cytochrome P-450 reductase (Butler and Hoey, 1993; Powis and Appel, 1980) and cytochrome *b*₅ reductase (Powis and Appel, 1980) have also been reported. However, when a similar approach was attempted for NQO1, no correlation was found between reduction potential and rates of reduction by NQO1 for a series of naphthoquinones (Buffington *et al.*, 1989) or a series of aziridinylbenzoquinones (Gibson *et al.*, 1992a). In the naphthoquinone study (Buffington *et al.*, 1989), rates of reduction by NQO1 also did not correlate with octanol-water partition coefficients which further precludes the ability to predict substrate efficiency for NQO1. In view of these data, there are basically two approaches to identifying efficient substrates for NQO1. The first is an experimental approach using recombinant rat and human NQO1 (Ross *et al.*, 1994), whereas the second is to use the crystal structure of NQO1. The crystal structure of rat liver NQO1 has been determined to 2.1 Å resolution and has been published in preliminary form (Li *et al.*, 1995). Using the experimental approach several excellent substrates have been identified and assayed using purified rat and human recombinant NQO1 (Table III). Streptonigrin, MeDZQ and EO9 are rapidly reduced by both the rat and human forms of NQO1, and with the exception of EO9, the rates of reduction of the bioreductive agents by human recombinant NQO1 are between 20% and 30% of the rates of reduction by rat recombinant NQO1 (Beall *et al.*, 1995).

Chemosensitivity studies have also been carried out using streptonigrin, MeDZQ, EO9 and other bioreductive agents (Beall *et al.*, 1995). Two non-small-cell lung cancer cell lines were used: H460 which has high NQO1 activity ($1360 \pm 100 \text{ nmol min}^{-1} \text{mg}^{-1}$) and H596 which has undetectable NQO1 activity. Selectivity ratios (SRs) were calculated as the concentration at which survival equals 50% of control (IC_{50}) for H596 cells divided by the IC_{50} for H460 cells. A higher SR implies a greater selectivity for cells with elevated NQO1 activity. In this system, streptonigrin was the most selective of the bioreductive drugs that were tested (SR = 86) followed by EO9 (SR = 62) and MeDZQ (SR = 17). This is in agreement with the ability of streptonigrin to serve as the most efficient substrate for NQO1 that we have tested to date (Beall *et al.*, 1995). In addition, streptonigrin showed the best correlation between NQO1 activity and cytotoxicity of over 31 000 compounds tested in the NCI human tumour cell line panel (Paull *et al.*, 1994).

It seems clear that compounds which are efficient substrates for bioreductive enzymes will be identified either on the basis of reduction potentials, experimental studies using purified enzymes or using crystal structures. Whether compounds should be directed against a single reductase such as NQO1 or whether they should be targeted to multiple reductases is an important question. In addition, a critical step in identifying appropriate compounds which should progress to the clinic is the selection of appropriate and predictive preclinical models.

In this regard, xenografts (Langdon *et al.*, 1994) and perhaps tumour spheroids may be more predictive models than simply using cell lines (Figure 6).

Potential problems with the bioreductive approach

Although NQO1 appears to be a useful target for bioreductive drug research, there are many potential problems with the bioreductive approach (Table IV). Tumour heterogeneity could be considered a fundamental flaw in the bioreductive approach since there may be marked differences in bioreductive enzymes in different cells within the tumour. Differences in oxygen tension, blood flow and pH may also modulate bioreductive enzyme activity within a tumour. For example, hypoxia has been shown to modulate enzyme activity by transcriptional induction and increased stability of mRNA (O'Dwyer *et al.*, 1994). In addition, a study using multicellular spheroids demonstrated that NQO1 activity and gene expression increased with increasing depth within the spheroid. This trend was reversed, however, in layers near the necrotic centre of the spheroid (Phillips *et al.*, 1994). Differences in cellular pH within a tumour can affect the activation of bioreductive drugs such as mitomycin C (Kennedy *et al.*, 1985; Pan and Hipsher, 1993; Siegel *et al.*, 1993). Environmental factors must also be considered since xenobiotics and dietary constituents can influence bioreductive enzyme activity. NQO1 and other detoxification enzymes are induced by a wide variety of chemical agents including bifunctional enzyme inducers (dioxin, polycyclic aromatic hydrocarbons, azo dyes, flavonoids) and monofunctional enzyme inducers (diphenols, isothiocyanates, dithiolthiones and thiocarbamates) (Prochaska *et al.*, 1985; Prochaska and Talalay, 1988). An important consideration is the effect that cigarette-smoking history has on bioreductive enzyme levels. Large differences in NQO1 activity have been reported between non-smokers, past smokers and present smokers in normal and tumour tissues in the colon and lung with the most dramatic differences seen in lung tumour tissues (Schlager and Powis, 1990). Elevated enzyme activities in normal tissues such as kidney (Schlager and Powis, 1990) may also be problematic and could potentially lead to unwanted bioreductive drug toxicity.

Many of the early studies in bioreductive drug research were carried out using reductases and cell lines of rodent origin. Extrapolation of data obtained using rodent models to human systems could be misleading. For example, rodent enzymes may catalyse bioreduction at very different rates than enzymes of

human origin (Table III). Work with CB1954 in rodent cell lines revealed another potential problem with rodent/human extrapolations. CB1954 was found to be between 500 and 5000 times less toxic to human cell lines than to rat cell lines with similar NQO1 activities (Boland *et al.*, 1991). The availability of purified recombinant human proteins, human tumour cell lines and xenografts should facilitate the design of future studies using human systems.

Finally, the presence of a genetic polymorphism in the NQO1 gene, and possibly other reductase genes, may necessitate implementation of pretreatment biopsies to determine if a functional bioreductive enzyme is present in the target tumour (Ross *et al.*, 1994). The absence of functional NQO1 activity in the BE colon carcinoma cell line led to the discovery of a C to T point mutation at position 609, exon 6 of the NQO1 cDNA which codes for a proline to serine change at position 187 of the NQO1 protein (Traver *et al.*, 1992). The same mutation has subsequently been identified in the H596 non-small-cell lung cancer cell line which also lacks detectable NQO1 activity (Traver *et al.*, 1995). These two cell lines have abundant NQO1 mRNA, but they are not immunoreactive to a monoclonal antibody to human NQO1. Since the same monoclonal antibody is immunoreactive to recombinant mutant NQO1 (Siegel *et al.*, 1995), this suggests that the NQO1 mutant protein is either not made, or it is made and rapidly degraded. The use of PCR to determine enzyme expression before bioreductive drug treatment has been suggested as a potential approach to individual patient enzyme profiling. The NQO1 polymorphism would present a problem in this regard since elevated mRNA expression, detected using PCR, would not determine the presence of the mutation. The incidence of the polymorphism in the NQO1 gene is as yet unknown, but one early study showed that NQO1 was absent in 4% of samples from a British population (Edwards *et al.*, 1980). Since the point mutation was discovered, Rosvold *et al.* (1993) determined that the mutation had a frequency of 0.11 in a reference population and noted that it was significantly overrepresented in lung cancer. An absence of NQO1 was also identified in approximately 10% of renal cancer and associated normal tissue (Eickelmann *et al.*, 1994). As previously discussed, NQO1 is classified as a detoxification and cancer-protective enzyme and whether a lack of NQO1 activity predisposes to cancer is an important question (Marshall *et al.*, 1991b). Work is currently underway to document the frequency and ethnic distribution of this potentially important polymorphism and its role, if any, in predisposition to cancer.

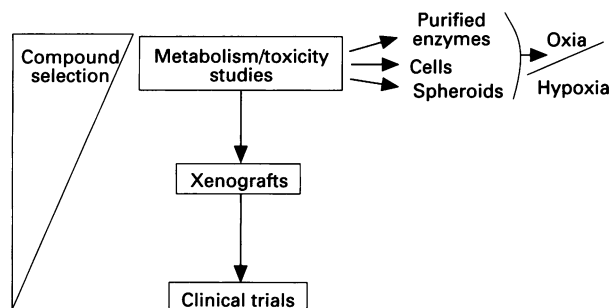


Figure 6 Possible test systems for bioreductive drug development.

Table IV Potential problems with the bioreductive approach

Tumour heterogeneity.
Elevated enzyme activities in normal tissues.
Influence of environmental factors on bioreductive enzymes.
Rodent/human differences.
Polymorphisms in bioreductive enzymes.

Conclusions

1. Present bioreductives are activated by multiple reductases, via both one- and two-electron reduction.
2. Some reductases, such as NQO1, are elevated in certain tumours, but there is a wide variation even within tumour groups.
3. Relationships between NQO1 activity and aerobic drug sensitivity have been reported for EO9 and mitomycin C.
4. Metabolism by NQO1 is oxygen insensitive. Increased toxicity of bioreductive quinones under hypoxia is mediated by other enzymes, presumably one-electron reductases.
5. NQO1 has been reported to protect cells from the hypoxic toxicity of EO9.
6. Potential problems with the enzyme-directed approach include tumour heterogeneity, elevated enzyme activities in normal tissue, influence of environmental factors on bioreductive enzymes, rodent/human differences and polymorphisms in bioreductive enzymes.

Future directions

1. Characterise reactive species generated, how they are produced and how they react under aerobic and hypoxic conditions.
2. Determine whether the protective effect of NQO1 against the hypoxic toxicity of EO9 extends to other bioreductive drugs and *in vivo* situations.
3. Transfection studies in human cell systems should be performed with human cDNAs.
4. Exploit the NCI database to identify new targets. Currently, the NCI panel only performs toxicity assays under aerobic conditions and selected assays should also be performed under hypoxia.
5. Incorporate parameters other than bioactivation such as DNA repair into predictive models.
6. Determine the incidence of polymorphisms in key enzymes such as NQO1 in different populations.
7. Use more predictive preclinical assays. Different test systems such as xenografts should be incorporated into the testing paradigm.

References

- ALLEY SC, BRAMELD KA AND HOPKINS PB. (1994). DNA interstrand cross-linking by 2,5-bis(1-aziridinyl)-1,4-benzoquinone: nucleotide sequence preferences and covalent structure of the dG-to-dG cross-links at 5'-d(GNnC) in synthetic oligonucleotide duplexes. *J. Am. Chem. Soc.*, **116**, 2734–2741.
- BACHUR NR, GORDON SL, GEE MV AND KON H. (1979). NADPH cytochrome P-450 reductase activation of quinone anticancer agents to free radicals. *Proc. Natl Acad. Sci. USA*, **76**, 954–957.
- BEALL HD, MULCAHY RT, SIEGEL D, TRAVER RD, GIBSON NW AND ROSS D. (1994). Metabolism of bioreductive antitumor quinones by purified rat and human DT-diaphorases. *Cancer Res.*, **54**, 3196–3201.
- BEALL HD, MURPHY AM, SIEGEL D, HARGREAVES RHJ, BUTLER J AND ROSS D. (1995). NAD(P)H:quinone oxidoreductase (DT-diaphorase) as a target for bioreductive antitumor quinones. Quinone cytotoxicity and selectivity in human lung and breast cancer cell lines. *Mol. Pharmacol.*, in press.
- BEGLEITER A, LEITH M, MCCLARTY G, BENNKEN S, GOLDBENBERG GJ AND WRIGHT JA. (1988). *Cancer Res.*, **48**, 1727–1735.
- BEGLEITER A, ROBOTHAM E, LACEY G AND LEITH MK. (1989). Increased sensitivity of quinone resistant cells to mitomycin C. *Cancer Lett.*, **45**, 173–176.
- BELCOURT MF, HODNICK WF, KEMPLE B, ROCKWELL S AND SARTORELLI AC. (1995). Induction of differential toxicity to mitomycin antibiotics by overexpression of NADPH:cytochrome c (P-450) reductase in Chinese hamster ovary (CHO) cells. *Proc. Am. Assoc. Cancer Res.*, **36**, 602.
- BERGER MS, TALCOTT RE, ROSENBLUM ML, SILVA M, ALI-OSMAN F AND SMITH MT. (1985). The use of quinones in brain tumor chemotherapy. Preliminary results from preclinical investigations. *J. Toxicol. Environ. Health*, **16**, 713–719.
- BLIGH HFJ, BARTOSZEK A, ROBSON CN, HICKSON ID, KASPER CB, BEGGS JD AND WOLF CR. (1990). Activation of mitomycin C by NADPH:cytochrome P-450 reductase. *Cancer Res.*, **50**, 7789–7792.
- BOLAND MP, KNOX RJ AND ROBERTS JJ. (1991). The differences in kinetics of rat and human DT-diaphorase result in differential sensitivity of derived cell lines to CB1954 (5-(aziridin-1-yl)-2,4-dinitrobenzamide). *Biochem. Pharmacol.*, **41**, 867–875.
- BUFFINGTON GD, OLLINGER K, BRUNMARK A AND CADENAS E. (1989). DT-diaphorase-catalysed reduction of 1,4-naphthoquinone derivatives and glutathionyl-quinone conjugates. *Biochem. J.*, **257**, 561–571.
- BUTLER J AND HOEY BM. (1993). The one-electron reduction potential of several substrates can be related to their reduction rates by cytochrome P-450 reductase. *Biochim. Biophys. Acta*, **1161**, 73–78.
- CADENAS E. (1995). Antioxidant and prooxidant functions of DT-diaphorase in quinone metabolism. *Biochem. Pharmacol.*, **49**, 127–140.
- CLARKE ED, GOULDING KH AND WARDMAN P. (1982). Nitroimidazoles as anaerobic electron acceptors for xanthine oxidase. *Biochem. Pharmacol.*, **31**, 3237–3242.
- CLARKE ED, WARDMAN P AND GOULDING KH. (1980). Anaerobic reduction of nitroimidazoles by reduced flavin mononucleotide and by xanthine oxidase. *Biochem. Pharmacol.*, **29**, 2684–2687.
- CONE R, HASAN SK, LOWN JW AND MORGAN AR. (1976). The mechanism of the degradation of DNA by streptonigrin. *Can. J. Biochem.*, **54**, 219–223.
- CORRAN HS, GREEN DE AND STRAUB FB. (1939). On the catalytic function of heart flavoprotein. *Biochem. J.*, **33**, 793–801.
- CRESTEIL T AND JAISWAL AK. (1991). High levels of expression of the NAD(P)H:quinone oxidoreductase (NQO1) gene in tumor cells compared to normal cells of the same origin. *Biochem. Pharmacol.*, **42**, 1021–1027.
- CROOKE ST AND BRADNER WT. (1976). Mitomycin C: a review. *Cancer Treat. Rev.*, **3**, 121–139.
- DEWAN JG AND GREEN DE. (1938). Coenzyme factor – a new oxidation catalyst. *Biochem. J.*, **32**, 626–639.
- DULHANTY AM AND WHITMORE GF. (1991). Chinese hamster ovary cell lines resistant to mitomycin C under aerobic but not hypoxic conditions are deficient in DT-diaphorase. *Cancer Res.*, **51**, 1860–1865.
- EDWARDS YH, POTTER J AND HOPKINSON DA. (1980). Human FAD-dependent NAD(P)H diaphorase. *Biochem. J.*, **187**, 429–436.

Key questions

1. Should we be developing 'single-enzyme' agents or continue with our present 'multiple enzyme' compounds?
2. In view of the potential problems, should we even be attempting the bioreductive approach without patient profiling?
3. How are bioreductive enzymes regulated in normal and tumour tissue?
4. Do these compounds work?

Acknowledgements

The authors are extremely grateful to Drs NW Gibson, J Butler and RM Phillips for their assistance and critical input. This work was supported by HHS R01 CA51210.



- EICKELMANN P, EBERT T, WARSKULAT U, SCHULZ WA AND SIES H. (1994). Expression of NAD(P)H:quinone oxidoreductase and glutathione S-transferases α and π in human renal cell carcinoma and in kidney cancer-derived cell lines. *Carcinogenesis*, **15**, 219–225.
- ERNSTER L. (1967). DT-diaphorase. *Methods Enzymol.*, **10**, 309–317.
- FITZSIMMONS SA, WORKMAN P, GREVER M, PAULL K, CAMALIER R AND LEWIS AD. (1996). The differential expression of reductase enzymes in the NCI human tumor cell line panel: correlation with sensitivity to mitomycin C and the investigational indoloquinone EO9. *J. Natl Cancer Inst.*, in press.
- FORREST GL, AKMAN S, DOROSHOW J, RIVERA H AND KAPLAN WD. (1991). Genomic sequence and expression of a cloned human carbonyl reductase gene with daunorubicin reductase activity. *Mol. Pharmacol.*, **40**, 502–507.
- GIBSON NW, HARTLEY JA, BUTLER J, SIEGEL D AND ROSS D. (1992a). Relationship between DT-diaphorase-mediated metabolism of a series of aziridinylbenzoquinones and DNA damage and cytotoxicity. *Mol. Pharmacol.*, **42**, 531–536.
- GIBSON NW, SIEGEL D AND ROSS D. (1992b). Mitomycin C. In *Cancer Chemotherapy and Biological Response Modifiers Annual 13*. Pinedo HM, Longo DL and Chabner BA. (eds) Chapter 5. Elsevier: Amsterdam.
- GUSTAFSON DL AND PRITSOS CA. (1992a). Bioactivation of mitomycin C by xanthine dehydrogenase from EMT6 mouse mammary carcinoma tumors. *J. Natl Cancer Inst.*, **84**, 1180–1185.
- GUSTAFSON DL AND PRITSOS CA. (1992b). Enhancement of xanthine dehydrogenase mediated mitomycin C metabolism by dicumarol. *Cancer Res.*, **52**, 6936–6939.
- HOBAN PR, WALTON MI, ROBSON CN, GODDEN J, STRATFORD IJ, WORKMAN P, HARRIS AL AND HICKSON ID. (1990). Decreased NADPH:cytochrome P-450 reductase activity and impaired drug activation in a mammalian cell line resistant to mitomycin C under aerobic but not hypoxic conditions. *Cancer Res.*, **50**, 4692–4697.
- HODNICK WF, BELCOURT MF, KEMPLE B, ROCKWELL S AND SARTORELLI AC. (1995). Potentiation of mitomycin C and porfiromycin toxicity to Chinese hamster ovary (CHO) cells by overexpression of DT-diaphorase (DTD) cDNA. *Proc. Am. Assoc. Cancer Res.*, **36**, 602.
- HODNICK WF AND SARTORELLI AC. (1993). Reductive activation of mitomycin C by NADH: cytochrome b_5 reductase. *Cancer Res.*, **53**, 4907–4912.
- HUANG MT, MIWA GT, CRONHEIM N AND LU AYH. (1979). Rat liver cytosolic azoreductase. *J. Biol. Chem.*, **254**, 11223–11227.
- IYANAGI T AND YAMAZAKI I. (1970). Difference in the mechanism of quinone reduction by the NADH dehydrogenase and the NAD(P)H dehydrogenase (DT-diaphorase). *Biochim. Biophys. Acta*, **216**, 282–294.
- IYER VN AND SZYBALSKI W. (1964). Mitomycins and porfiromycin: chemical mechanism of activation and cross-linking of DNA. *Science*, **145**, 55–58.
- JAISWAL AK. (1994). Human NAD(P)H:quinone oxidoreductase2. Gene structure, activity, and tissue-specific expression. *J. Biol. Chem.*, **269**, 14502–14508.
- JAISWAL AK, MCBRIDE OW, ADESNIK M AND NEBERT DW. (1988). Human dioxin-inducible cytosolic NAD(P)H:menadiione oxidoreductase. *J. Biol. Chem.*, **263**, 13572–13578.
- JAISWAL AK, BURNETT P, ADESNIK M AND MCBRIDE OW. (1990). Nucleotide and deduced amino acid sequence of a human cDNA (NQO₂) corresponding to a second member of the NAD(P)H:quinone oxidoreductase gene family. Extensive polymorphism at the NQO₂ gene locus on chromosome 6. *Biochemistry*, **29**, 1899–1906.
- KENNEDY KA, MCGURL JD, LEONARIDIS L AND ALABASTER O. (1985). pH dependence of mitomycin C-induced cross-linking activity in EMT6 tumor cells. *Cancer Res.*, **45**, 3541–3547.
- KEYES SR, FRACASSO PM, HEIMBROOK DC, ROCKWELL S, SLIGAR SG AND SARTORELLI AC. (1984). Role of NADPH:cytochrome c reductase and DT-diaphorase in the bioactivation of mitomycin C. *Cancer Res.*, **44**, 5638–5643.
- KEYES SR, ROCKWELL S AND SARTORELLI AC. (1985). Enhancement of mitomycin C cytotoxicity to hypoxic tumor cells by dicoumarol in vivo and in vitro. *Cancer Res.*, **45**, 213–216.
- KEYES SR, ROCKWELL S AND SARTORELLI AC. (1989). Modification of the metabolism and cytotoxicity of bioreductive alkylating agents by dicoumarol in aerobic and hypoxic murine tumor cells. *Cancer Res.*, **49**, 3310–3313.
- KNOX RJ, BOLAND MP, FRIEDLOS F, COLES B, SOUTHAN C AND ROBERTS JJ. (1988). The nitroreductase enzyme in Walker cells that activates 5-(aziridin-1-yl)-2,4-dinitrobenzamide (CB 1954) to 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide is a form of NAD(P)H dehydrogenase (quinone) (EC 1.6.99.2). *Biochem. Pharmacol.*, **37**, 4671–4677.
- KNOX RJ, FRIEDLOS F, MARCHBANK T AND ROBERTS JJ. (1991). Bioactivation of CB 1954: reaction of the active 4-hydroxylamino derivative with thioesters to form the ultimate DNA–DNA interstrand crosslinking species. *Biochem. Pharmacol.*, **42**, 1691–1697.
- KOMIYAMA T, KIKUCHI T AND SUGIURA Y. (1982). Generation of hydroxyl radical by anticancer quinone drugs, carbaziquinone, mitomycin C, aclacinomycin A and adriamycin, in the presence of NADPH-cytochrome P-450 reductase. *Biochem. Pharmacol.*, **31**, 3651–3656.
- LADEROUTE K, WARDMAN P AND RAUTH AM. (1988). Molecular mechanism for the hypoxia-dependent activation of 3-amino-1,2,4-benzotriazine-1,4-dioxide. *Biochem. Pharmacol.*, **37**, 1487–1495.
- LANGDON SP, HENDRIKS HR, BRAAKHUIS BJM, PRATESI G, BERGER DP, FODSTAD O, FIEBIG HH AND BOVEN E. (1994). *Ann. Oncol.*, **5**, 415–422.
- LEE CS, HARTLEY JA, BERARDINI MD, BUTLER J, SIEGEL D, ROSS D AND GIBSON NW. (1992). Alteration in DNA cross-linking and sequence selectivity of a series of aziridinylbenzoquinones after enzymatic reduction by DT-diaphorase. *Biochemistry*, **31**, 3019–3025.
- LI R, BIANCHET M, TALALAY P AND AMZEL LM. (1995). The three dimensional structure of NAD(P)H:quinone acceptor oxidoreductase, a chemoprotective flavoprotein, determined to 2.1 Å resolution. *FASEB J.*, **9**, A1338.
- LIN AJ, COSBY LA, SHANSKY CW AND SARTORELLI AC. (1972). Potential bioreductive alkylating agents. 1. Benzoquinone derivatives. *J. Med. Chem.*, **15**, 1247–1252.
- LIND C, CADENAS E, HOCHSTEIN P AND ERNSTER L. (1990). DT-diaphorase: purification, properties and function. *Methods Enzymol.*, **186**, 287–301.
- MALIEPAARD M, WOLFS A, GROOT SE, DE MOL NJ AND JANSSEN LHM. (1995). Indoloquinone EO9: DNA interstrand cross-linking upon reduction by DT-diaphorase or xanthine oxidase. *Br. J. Cancer*, **71**, 836–839.
- MALKINSON AM, SIEGEL D, FORREST GL, GAZDAR AF, OIE HK, CHAN DC, BUNN PA, MABRY M, DYKES DJ, HARRISON JR SD AND ROSS D. (1992). Elevated DT-diaphorase activity and messenger RNA content in human non-small cell lung carcinoma: relationship to the response of lung tumor xenografts to mitomycin C. *Cancer Res.*, **52**, 4752–4757.
- MARSHALL RS, PATERSON MC AND RAUTH AM. (1989). Deficient activation by a human cell strain leads to mitomycin resistance under aerobic but not hypoxic conditions. *Br. J. Cancer*, **59**, 341–346.
- MARSHALL RS, PATERSON MC AND RAUTH AM. (1991a). Studies on the mechanism of resistance to mitomycin C and porfiromycin in a human cell strain derived from a cancer-prone individual. *Biochem. Pharmacol.*, **41**, 1351–1360.
- MARSHALL RS, PATERSON MC AND RAUTH AM. (1991b). DT-diaphorase activity and mitomycin C sensitivity in non-transformed cell strains derived from members of a cancer-prone family. *Carcinogenesis*, **12**, 1175–1180.
- O'DWYER PJ, YAO KS, FORD P, GODWIN AK AND CLAYTON M. (1994). Effects of hypoxia on detoxicating enzyme activity and expression in HT29 colon carcinoma cells. *Cancer Res.*, **54**, 3082–3087.
- PAN SS, ANDREWS PA, GLOVER CJ AND BACHUR NR. (1984). Reductive activation of mitomycin C and mitomycin C metabolites catalysed by NADPH:cytochrome P-450 reductase and xanthine oxidase. *J. Biol. Chem.*, **259**, 959–966.
- PAN SS, YU F AND HIPHER C. (1993). Enzymatic and pH modulation of mitomycin C-induced DNA damage in mitomycin C-resistant HCT 116 human colon cancer cells. *Mol. Pharmacol.*, **43**, 870–877.
- PATTERSON AV, ROBERTSON N, HOULBROOK S, STEPHENS MA, ADAMS GE, HARRIS AL, STRATFORD IJ AND CARMICHAEL J. (1994). The role of DT-diaphorase in determining the sensitivity of human tumor cells to tirapazamine (SR 4233). *Int. J. Radiat. Oncol. Biol. Phys.*, **29**, 369–372.

- PAULL K, CAMALIER R, FITZSIMMONS SA, LEWIS AD, WORKMAN P AND GREVER M. (1994). Correlations of DT-diaphorase expression with cell sensitivity data obtained from the NCI human tumor cell line panel. *Proc. Am. Assoc. Cancer Res.*, **35**, 369.
- PHILLIPS RM, DE LA CRUZ, A, TRAVER RD AND GIBSON NW. (1994). Increased activity and expression of NAD(P)H:quinone acceptor oxidoreductase in confluent cell cultures and within multicellular spheroids. *Cancer Res.*, **54**, 3766–3771.
- PLUMB JA AND WORKMAN P. (1994). Unusually marked hypoxic sensitisation to indoloquinone EO9 and mitomycin C in a human colon-tumour cell line that lacks DT-diaphorase activity. *Int. J. Cancer*, **56**, 134–139.
- PLUMB JA, GERRITSEN M AND WORKMAN P. (1994). DT-diaphorase protects cells from the hypoxic cytotoxicity of indoloquinone EO9. *Br. J. Cancer*, **70**, 1136–1143.
- POWIS G AND APPEL PL. (1980). Relationship of the single-electron reduction potential of quinones with their reduction by flavoproteins. *Biochem. Pharmacol.*, **29**, 2567–2572.
- PROCHASKA HJ AND TALALAY P. (1988). Regulatory mechanisms of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver. *Cancer Res.*, **48**, 4776–4782.
- PROCHASKA HJ, DE LONG MJ AND TALALAY P. (1985). On the mechanisms of induction of cancer-protective enzymes: a unifying proposal. *Proc. Natl Acad. Sci. USA*, **82**, 8232–8236.
- ROBERTSON N, STRATFORD IJ, HOULBROOK S, CARMICHAEL J AND ADAMS GE. (1992). The sensitivity of human tumour cells to quinone bioreductive drugs: what role for DT-diaphorase? *Biochem. Pharmacol.*, **44**, 409–412.
- ROBERTSON N, HAIGH A, ADAMS GE AND STRATFORD IJ. (1994). Factors affecting sensitivity to EO9 in rodent and human tumour cells in vitro: DT-diaphorase activity and hypoxia. *Eur. J. Cancer*, **30A**, 1013–1019.
- ROSS D, SIEGEL D, BEALL H, PRAKASH AS, MULCAHY RT AND GIBSON NW. (1993). DT-diaphorase in activation and detoxification of quinones. *Cancer Metastasis Rev.*, **12**, 83–101.
- ROSS D, BEALL H, TRAVER RD, SIEGEL D, PHILLIPS RM AND GIBSON NW. (1994). Bioactivation of quinones by DT-diaphorase. Molecular, biochemical and chemical studies. *Oncol. Res.*, **6**, 493–500.
- ROSVOLD EA, MCGLYNN KA, LUSTBADER ED AND BUETOW KH. (1993). Identification of an NAD(P)H:quinone oxidoreductase polymorphism and its association with lung cancer. *Proc. Am. Assoc. Cancer Res.*, **34**, 144.
- SARTORELLI AC, HODNICK WF, BELCOURT MF, TOMASZ M, HAFFTY B, FISCHER JJ AND ROCKWELL S. (1994). Mitomycin C: a prototype bioreductive agent. *Oncol. Res.*, **6**, 501–508.
- SCHLAGER JJ AND POWIS G. (1990). Cytosolic NAD(P)H:quinone acceptor oxidoreductase in human normal and tumor tissue. Effects of cigarette smoking and alcohol. *Int. J. Cancer*, **45**, 403–409.
- SHIBATA K, KASAHARA K, BANDO T, NAKATSUMI Y, FUJIMURA M, TSURUO T AND MATSUDA T. (1995). Establishment and characterization of non-small cell lung cancer cell lines resistant to mitomycin C under aerobic conditions. *Jpn. J. Cancer Res.*, **86**, 460–469.
- SIEGEL D, GIBSON NW, PREUSCH PC AND ROSS D. (1990). Metabolism of mitomycin C by DT-diaphorase: role in mitomycin C-induced DNA damage and cytotoxicity in human colon carcinoma cells. *Cancer Res.*, **50**, 7483–7489.
- SIEGEL D, BEALL H, SENEKOWITSCH C, KASAI M, ARAI H, GIBSON NW AND ROSS D. (1992). Bioreductive activation of mitomycin C by DT-diaphorase. *Biochemistry*, **31**, 7879–7885.
- SIEGEL D, BEALL H, KASAI M, ARAI H, GIBSON NW AND ROSS D. (1993). pH-dependent inactivation of DT-diaphorase by mitomycin C and porfiromycin. *Mol. Pharmacol.*, **44**, 1128–1134.
- SIEGEL D, TRAVER RD, BEALL HD AND ROSS D. (1995). Cloning and purification of a mutant DT-diaphorase protein from human colon and lung cancer cell lines. *Proc. Am. Assoc. Cancer Res.*, **36**, 291.
- SMITSKAMP-WILMS E, PETERS GJ, PINEDO HM, VAN ARK-OTTE J AND GIACCONE G. (1994). Chemosensitivity to the indoloquinone EO9 is correlated with DT-diaphorase activity and its gene expression. *Biochem. Pharmacol.*, **47**, 1325–1332.
- SPAIN RC. (1993). The case for mitomycin in non-small cell lung cancer. *Oncology*, **50**(suppl. 1), 35–52.
- TRAVER RD, HORIKOSHI T, DANENBERG KD, STADLBAUER THW, DANENBERG PV, ROSS D AND GIBSON NW. (1992). NAD(P)H:quinone oxidoreductase gene expression in human colon carcinoma cells: characterization of a mutation which modulates DT-diaphorase activity and mitomycin sensitivity. *Cancer Res.*, **52**, 797–802.
- TRAVER RD, PHILLIPS RM, GIBSON NW AND ROSS D. (1995). A point mutation in both human lung and colon carcinoma cell lines leading to a loss of DT-diaphorase activity. *Proc. Am. Assoc. Cancer Res.*, **36**, 525.
- WALTON MI AND WORKMAN P. (1993). Pharmacokinetics and bioreductive metabolism of the novel benzotriazine di-N-oxide hypoxic cell cytotoxin tirapazamine (WIN 59075; SR 4233; NSC 130181) in mice. *J. Pharmacol. Exp. Ther.*, **265**, 938–947.
- WALTON MI, SMITH PJ AND WORKMAN P. (1991). The role of NAD(P)H:quinone reductase (EC 1.6.99.2, DT-diaphorase) in the reductive bioactivation of the novel indoloquinone antitumor agent EO9. *Cancer Commun.*, **3**, 199–206.
- WORKMAN P. (1994). Enzyme directed bioreductive drug development revisited: A commentary on recent progress and future prospects with emphasis on quinone anticancer agents and quinone metabolizing enzymes, particularly DT-diaphorase. *Oncol. Res.*, **6**, 461–475.
- WORKMAN P AND WALTON MI. (1990). Enzyme-directed bioreductive drug development. In *Selective Activation of Drugs by Redox Processes*, Adams GE, Breccia A, Fielden EM and Wardman P. (eds) pp. 173–191. Plenum: New York.
- WORKMAN P, WALTON MI, BAILEY SM AND SUGGETT NR. (1991). Enzyme-directed bioreductive drug development: molecular features which favour activation of quinone bioreductives by DT-diaphorase. *Br. J. Cancer*, **64**(suppl. 15), 4.