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Review

NAD(P)H:quinone oxidoreductase 1 (NQO1) in the sensitivity and resistance to antitumor quinones

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ABSTRACT

Quinones represent a large and diverse class of antitumor drugs and many quinones are approved for clinical use or are currently undergoing evaluation in clinical trials. For many quinones reduction to the hydroquinone has been shown to play a key role in their antitumor activity. The two-electron reduction of quinones by NQO1 has been shown to be an efficient pathway to hydroquinone formation. NQO1 is expressed at high levels in many human solid tumors making this enzyme ideally suited for intracellular drug activation. Cellular levels of NQO1 are influenced by the NQO1*2 polymorphism. Individuals homozygous for the NQO1*2 allele are NQO1 null and homozygous NQO1*2*2 cell lines have been shown to be more resistant to antitumor quinones when compared to isogenic cell lines overexpressing NQO1. In this review we will discuss the role of NQO1 in the sensitivity and resistance of human cancers to the quinone antitumor drugs mitomycin C, β-lapachone and the benzoquinone ansamycin class of Hsp90 inhibitors including 17-AAG. The role of NQO1 in the bioreductive activation of mitomycin C remains controversial but pre-clinical data strongly suggests a role for NQO1 in the activation of β-lapachone and the benzoquinone ansamycin class of Hsp90 inhibitors. Despite a large volume of preclinical data demonstrating that NQO1 is an important determinant of sensitivity to these antitumor quinones there is little information on whether the clinical response to these agents is influenced by the NQO1*2 polymorphism. The availability of simple assays for the determination of the NQO1*2 polymorphism should facilitate clinical testing of this hypothesis.

Contents

The quinone pharmacophore is present in many drug classes but is particularly common among antitumor drugs. Many quinones serve essentially as pro-drugs and exert their activities after reduction. Reduction of quinones may generate semiquinones or hydroquinones with subsequent generation of reactive oxygen radicals and oxidative stress, quinones can be designed so they lose a leaving group when reduced to the hydroquinone generating a reactive electrophile or the hydroquinone form of the molecule may have greater pharmacological activity than the parent quinone against a particular target. Enzyme systems that reduce quinones therefore become critically important in the pharmacological activity of this class of drugs. There are a number of enzyme systems that can catalyze reduction of quinones including NADPH cytochrome P450 reductase, NADH cytochrome b5 reductase, NAD(P)H:quinone oxidoreductase 1 (NQO1), NAD(P)H:quinone oxidoreductase 2 (NQO2), carbonyl reductases, and thioredoxin reductase. In this context, one of the most extensively studied reductases has been NAD(P)H:quinone oxidoreductase 1 (NQO1). In

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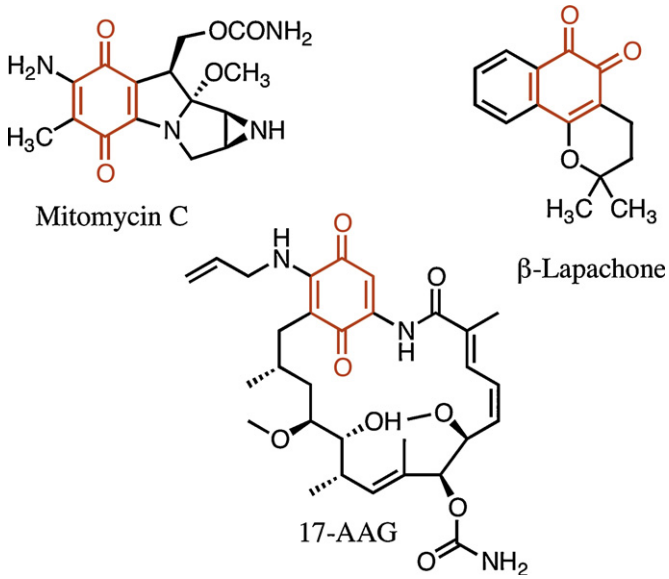

Fig. 1. Chemical structures of mitomycin C, β-lapachone and 17-AAG. The quinone moiety is highlighted in red. 17-AAG, 17-N-allylamino-17-demethoxygeldanamycin.

this review we will focus on the role of NQO1 in the bioactivation of clinically important quinones mitomycin C, β-lapachone and 17AAG (Fig. 1) as well as the influence of the NQO1*2 polymorphism on the sensitivity and resistance to these agents.

1. NQO1

NAD(P)H:quinone oxidoreductase 1 (NQO1, EC 1.6.99.2) is a flavoenzyme that catalyzes the two-electron reduction of quinones to their hydroquinone forms [1]. NQO1 functions as a homodimer with one FAD bound per monomer. This enzyme utilizes reduced pyridine nucleotide cofactors NADH or NADPH to catalyze the direct two-electron reduction of a broad range of quinones [2]. The crystal structure of human NQO1 was resolved in 2000 [3] and this work demonstrated that the cofactor and the substrate share the same binding site confirming the ping–pong mechanism of catalysis [4]. NQO1 is localized primarily in the cytosol but lower levels have been detected in the nucleus [5]. In human tissues NQO1 is expressed at high levels in many epithelial cells as well as vascular endothelium and adipocytes [6,7]. Humans, unlike most other mammals, do not express NQO1 in normal liver hepatocytes [6,8] but NQO1 expression is seen in pre-neoplastic lesions and liver cancers [9,10]. NQO1 is expressed at high levels in most human solid tumors including tumors from colon, breast, pancreas and lung [6,11].

There are two characterized polymorphisms in NQO1, NQO1*2 and NQO1*3, with well-defined phenotypes and frequencies. The NQO1*2 polymorphism is characterized by a C to T change at position 609 of the human cDNA which results in a proline to serine substitution at amino acid 187 of NQO1 [12]. The resulting mutant NQO1 protein is catalytically inactive due to the inability to correctly bind the FAD cofactor [13]. The mutant NQO1*2 protein has also been shown to bind to the Hsp70 binding domain of the E3 ubiquitin ligase STUB1/CHIP which catalyzes the ubiquitination of the NQO1*2 protein resulting in proteasomal degradation [14,15]. Individuals genotyped as homozygous for the NQO1*2 polymorphism are NQO1 null, while individuals genotyped as heterozygous have reduced levels of NQO1 activity and protein [16]. The allele frequency of the NQO1*2 polymorphism is much lower in Caucasians compared to Asian populations [17]. In some Asian populations the percentage of individuals homozygous for the NQO1*2 polymorphism can be as high as 40% [18,19]. The NQO1*3 polymorphism has been characterized as a C465T substitution resulting in an arginine to tryptophan amino acid change in the protein [20,21]. The variant NQO1*3 protein has similar stability to the wildtype NQO1*1 protein and is catalytically active but major differences in the two proteins in the rate of metabolism of quinone substrates have been observed [20]. The allele frequency of the NQO1*3 polymorphism ranges from >0.01 in Inuit population to 0.05 in Caucasians [17].

The high levels of expression of NQO1 in solid tumors in combination with the ability to reduced many quinone-containing antitumor drugs has drawn attention to NQO1 as a potential molecular target in cancer treatment (Fig. 1).

1.1. Bioreductive activation of quinones by NQO1

The direct two-electron reduction of quinones to hydroquinones by NQO1 is historically considered a detoxification mechanism because this reaction by-passes the formation of the highly reactive semiquinone. However, in reality whether the formation of the hydroquinone is a detoxification reaction, or alternatively, an activation reaction will depend upon the chemical reactivities of the quinone and hydroquinone. There are many examples of naturally occurring and synthetic quinones that following reduction to their corresponding hydroquinones induce toxicity. The ability of NQO1 to generate cytotoxic hydroquinones has been utilized as a strategy to combat antiproliferative diseases such as cancer. As shown in Fig. 2, a hydroquinone generated following reduction by NQO1 can exert toxicity through a number of mechanisms depending upon its chemical reactivity. Unstable hydroquinones can undergo chemical rearrangements leading to alkylation of essential biomolecules such as DNA or undergo redox reactions leading to the formation of highly reactive oxygen species. Alternatively, if the hydroquinone is chemically stable it may possess unique or enhanced pharmacological properties not observed with the parent quinone. As shown in Fig. 2 NQO1 has been implicated in the bioactivation of many antitumor quinones. In this review we will discuss the role of NQO1 is the bioactivation of three clinically significant quinones mitomycin C, β-lapachone and 17AAG.

1.2. Bioreductive activation of mitomycin C by NQO1

Mitomycin C (MMC) is a quinone containing antibiotic isolated from *Streptomyces caespitosus*. MMC has been used clinically for

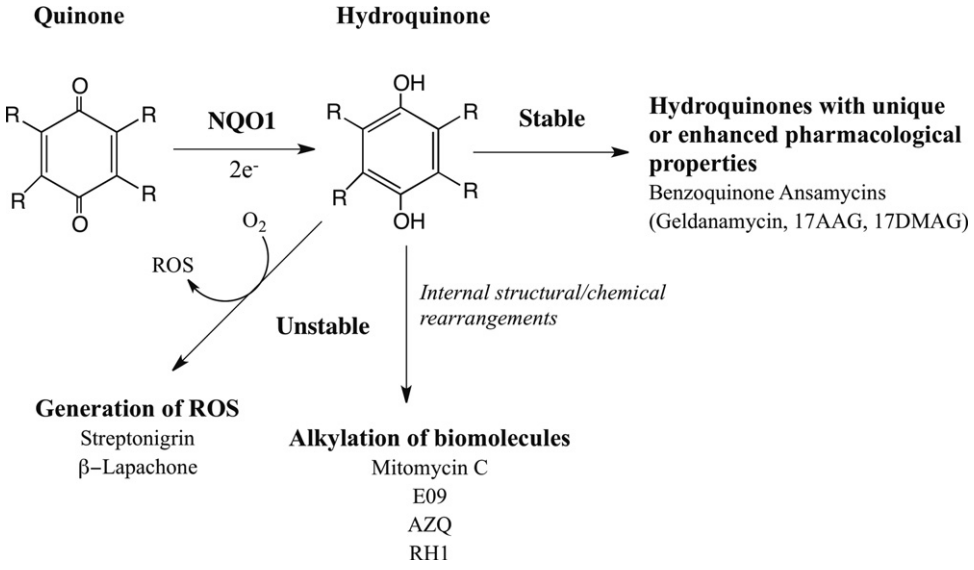

Fig. 2. Pathways for bioreductive activation of antitumor quinones by NQO1 (ROS, reactive oxygen species).

greater than 30 years for the treatment of solid tumors including stomach, pancreas, breast and lung. The mechanism of action of MMC is believed to be intracellular bioreductive activation leading to DNA interstrand crosslinking.

Studies using cultured tumor cell lines in combination with the NQO1 inhibitor dicumarol suggested a positive correlation between NQO1 (DT-diaphorase) catalytic activity and MMC sensitivity [22–41]. Under hypoxia, however, dicumarol potentiated MMC-induced DNA crosslinking and cytotoxicity suggesting that under hypoxic conditions MMC may be activated more efficiently by other bioreductive enzymes [22,42–45].

Experiments using purified rat and human NQO1 confirmed that these enzymes could bioactivate MMC, however, the metabolism of MMC by NQO1 was discovered to be pH-dependent [24,46]. When reactions were performed under acidic conditions MMC underwent bioactivation by NQO1 to a reactive species capable of crosslinking DNA as well as metabolites including 2,7-diaminomitosene [46]. However, as the pH of the reaction was increased MMC-induced DNA crosslinking and metabolite formation decreased substantially. Biochemical studies with purified NQO1 and MMC revealed that MMC was a pH-dependent mechanism-based inhibitor of NQO1 [47]. Under basic pH conditions NQO1 underwent alkylation by leucomitomycin C (MMC hydroquinone) in or near the active site of NQO1 resulting in the inhibition of catalytic activity. As the pH of the reaction was decreased inactivation of NQO1 by leucomitomycin became less efficient resulting in the release from the active site of leucomitomycin and subsequent alkylation of biomolecules such as DNA (Fig. 3A). Studies in cultured cells confirmed that under acidic conditions MMC induced greater levels of DNA crosslinking and more pronounced growth inhibition [30,40,48,49].

A role for NQO1 in MMC activation is supported by cell culture and xenograft experiments using isogenic cell lines engineered to overexpress NQO1. These studies demonstrated a positive correlation between NQO1 expression and sensitivity to MMC

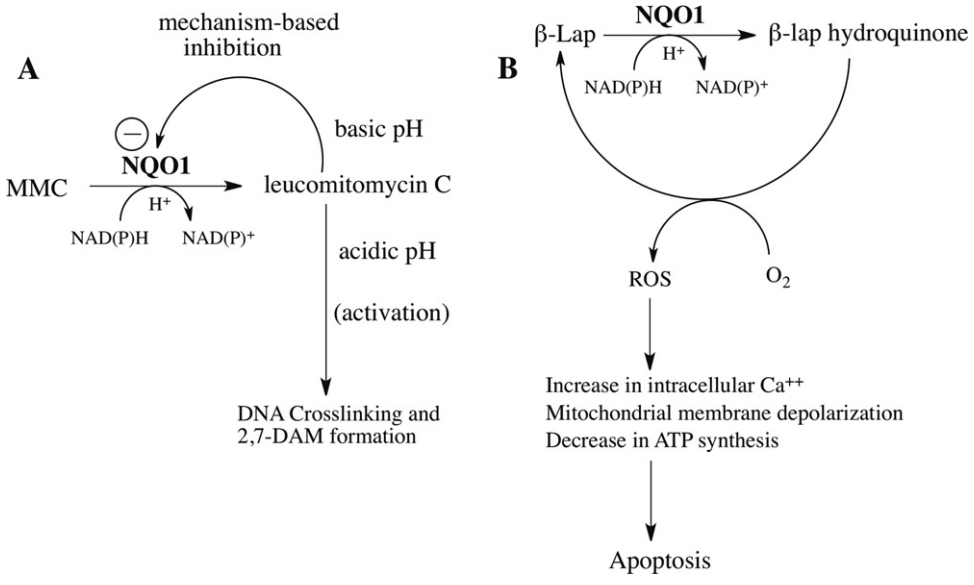

Fig. 3. The role of NQO1 in the bioactivation of mitomycin C and β-lapachone. (A) Reduction of MMC by NQO1 generates leucomitomycin C (MMC hydroquinone) which under basic conditions alkylates NQO1 in the active site preventing further metabolism. Under acidic conditions, the leucomitomycin C escapes the active site in NQO1 and can alkylate important biomolecules such as DNA or form the major metabolite 2,7-diaminomitosene (2,7-DAM) and (B) reduction of β-lapachone by NQO1 forms an unstable hydroquinone which interacts with molecular oxygen to generate reactive oxygen species leading to apoptosis.

[35,50–52]. Dietary induction of NQO1 by dimethylfumarate increased the sensitivity of human xenograft colon tumors to MMC [53]. In experiments where cultured cells were exposed to sub-lethal concentrations of MMC for extended periods of time resistant clones developed that were shown to have lower levels of NQO1 activity [31,35,54–61]. Genetic mutations in the NQO1 gene resulting in lower NQO1 protein levels and decreased catalytic activity were also seen in HCT116 human colon carcinoma cells made resistant to MMC by continuous low-dose exposure [20,27].

Studies that have examined the relationship between NQO1 protein expression/activity and MMC sensitivity in more complex systems such as mouse xenograft models of human cancers or in human subjects with cancers are limited. Sensitivity to MMC was shown to correlate with NQO1 activity in mouse xenograft model using human NSCLC [62] while no relationship between MMC sensitivity and the NQO1*2 allele (see above) was observed in a series of human tumor xenografts [63]. In patients with disseminated peritoneal cancer receiving intraperitoneal hyperthermic chemotherapy with MMC lower levels of NQO1 activity in dissected tumor tissues was associated with reduced survival in a subset of patients [64]. The widespread use of intravesical MMC therapy for the treatment of superficial bladder cancers has generated interest in a search for biomarkers of MMC sensitivity. The expression of NQO1 and NADPH cytochrome P450 reductase in a series of bladder tumors were shown to correlate with MMC sensitivity [65]. In another study, however, no correlation was observed between immunohistochemical staining for NQO1 or NADPH cytochrome P450 reductase in resected bladder tumors and clinical response to MMC [66]. In addition, genotyping of human bladder tumors for the NQO1*2 polymorphism was also found to be a poor predictor of a clinical response to MMC [67].

A major dose limiting toxicity observed with the clinical use of MMC is bone marrow depression [68]. NQO1 levels or the NQO1*2 polymorphism have not been previously associated with an increased risk of developing complications due to MMC therapy. In experiments using NQO1 knockout mice treated with MMC it was observed that mice deficient in NQO1 were resistant to MMC-induced bone marrow toxicity [69].

Biochemical and cell based experiments clearly demonstrate that NQO1 can bioactivate MMC and is generally a good predictor of MMC sensitivity. Given the multitude of factors that could influence the antitumor response to MMC including intracellular pH and O_2 concentrations, competing bioreductive enzymes, as well as DNA repair enzymes responsible for the repair of cytotoxic MMC-DNA interstrand crosslinks it is not surprising that NQO1 genotype or NQO1 protein levels by themselves may not be suitable candidates to predict clinical response to MMC therapy.

1.3. Bioactivation of β-lapachone by NQO1

β-Lapachone is a naturally occurring *ortho* napthoquinone isolated from the bark of the lapacho tree (*Tabebuia avellanedae*). β-lapachone was shown to have anti-bacterial and anti-fungal and anti-trypanosomal properties primarily due to the ability of β-lapachone to rapidly induce the formation of superoxide and hydrogen peroxide with the simultaneous oxidation of reduced pyridine nucleotides [70]. Early experiments demonstrated that β-lapachone could inhibit the repair of mammalian DNA through a mechanism involving inhibition of topoisomerase I [71–74]. β-lapachone has been shown to induce apoptosis in human leukemia and prostate cancer cells and over-expression of BCL2 could protect cells against β-lapachone induced apoptosis [75].

Studies in human breast and prostate cancer cell lines demonstrated that dicumarol could protect against β-lapachone-induced growth inhibition in NQO1-rich cells but had limited effect on NQO1-null cells implicating a role for NQO1 in β-lapachone toxicity [76,77]. In addition, in these studies the overexpression of NQO1 increased the sensitivity of breast and prostate cancer cells to β-lapachone [76,77]. Lysates prepared from breast cancer cells overexpressing NQO1 catalyzed the oxidation of NADH in the presence of β-lapachone. In these studies greater than 50 molar equivalents of NADH were oxidized per molar equivalent of β-lapachone suggesting that β-lapachone underwent NQO1-dependent redox cycling [76]. NQO1 catalyzes the redox cycling of β-lapachone through the generation of an unstable hydroquinone, which under aerobic conditions, is rapidly oxidized back to the parent quinone. Redox cycling of β-lapachone is characterized by the oxidation of large amounts of reduced pyridine nucleotides and the formation of reactive oxygen species including superoxide and hydrogen peroxide [78]. The reductive activation of β-lapachone by NQO1 (Fig. 3B) has been shown to result in a rapid increase in intracellular calcium leading to mitochondrial membrane depolarization, loss of ATP, DNA fragmentation and apoptosis [79,80].

In experiments where β-lapachone was used in combination with irradiation sensitivity to β-lapachone could be increased if cancer cells were irradiated in the presence of β-lapachone or if β-lapachone was added up to 24 h after irradiation suggesting that β-lapachone had a direct effect on DNA repair as well as effects that were independent of DNA repair [81,82]. The synergy observed between irradiation and β-lapachone when the drug is administered hours after irradiation could be explained by the upregulation of NQO1 [81,82]. X-ray irradiation has been shown to induce the expression of NQO1 in human cancer cells [83] and it has been proposed that at longer time points after irradiation the upregulation of NQO1 increased β-lapachone-dependent redox cycling leading to increased cytotoxicity. A similar mechanism has been proposed for the synergy observed between hyperthermia and β-lapachone where hyperthermia was shown to increase the expression of NQO1 in tumor cells resulting in greater sensitivity to β-lapachone [84–86].

The ability of NQO1 to efficiently catalyze the redox cycling of β-lapachone has been exploited to target human tumors with high levels of NQO1. Non-small cell lung cancers (NSCLC) have been shown to have high levels of NQO1 expression [7,11,62] and correspondingly NSCLC cells were shown to be very sensitive to β-lapachone. In these studies the sensitivity to β-lapachone was directly related to NQO1 protein levels [87]. NQO1 has been selected as a target for the activation of β-lapachone in pancreatic cancers since pancreatic cancers have significantly higher levels of NQO1 when compared to normal pancreatic tissue [88]. In human pancreatic cancer cells the cytotoxicity of β-lapachone was shown to be significantly decreased by dicumarol pretreatment and in experiments using siRNA to knockdown NQO1 protein levels [89]. In a more detailed study using the MiaPaCa-2 cell line in combination with shRNA targeted against NQO1 stable cell lines were created expressing varying levels of NQO1 [80]. In studies using these isogenic cell lines it was clear that there was a correlation between NQO1 activity and bioactivation of β-lapachone and it was concluded that a threshold level of NQO1 catalytic activity of 90U was required to efficiently activate β-lapachone in MiaPaCa-2 cells [80].

β-lapachone (ARQ 501) has entered Phase 1 and 2 clinical trials for the treatment of solid tumors. Despite the large volume of data implicating NQO1 in the bioactivation and cytotoxicity of β-lapachone, including many studies with human cell lines that are deficient in NQO1 due to the NQO1*2*2 genotype and were very resistant to β-lapachone, there are no published papers or abstracts from these clinical trials that have reported if sensitivity to β-lapachone is related to NQO1 activity and whether resistance to β-lapachone is observed in patients because they carry the NQO1*2*2 genotype.

1.4. Bioactivation of benzoquinone ansamycins by NQO1

The benzoquinone ansamycins (BQAs) including geldanamycin, 17-AAG and 17-DMAG are a group of quinone containing polyketide antibiotics. Geldanamycin (GA) was isolated from *Streptomyces hygroscopicus* [90,91] and GA was originally found to have antitumor properties due to its ability to inhibit RNA and DNA replication [92–94]. Later it was shown that GA could inhibit the expression of the oncogene cMyc [92] and inhibit the activity of vSrc [95,96]. Studies using GA affinity chromatography led to the discovery that heat shock protein 90 (Hsp90) was the target of GA [96]. BQAs bind to the ATP binding pocket in Hsp90 and inhibit the ATPase activity of the enzyme preventing the correct folding of newly synthesized proteins. Hsp90 has been shown to play a role in the maturation of many oncogenic proteins such as Raf-1 [97], HER2 [98,99], BCR-ABL [100], KIT [101,102] as well as steroid hormone receptors [103]. Hsp90 has become an attractive antitumor target since the inhibition of the chaperone function of Hsp90 can alter many key oncogenic pathways simultaneously. Preclinical studies with GA resulted in hepatotoxicity and less toxic analogs of GA including 17-AAG (17-N-allylamino-17-demethoxygeldanamycin) and 17-DMAG (17-(dimethylaminoethylamino)-17-demethoxygeldanamycin) were developed that focused on substitutions on 17-position on the ansamycin ring [104]. 17-AAG and 17-DMAG have shown antitumor activity against a wide spectrum of human cancers in laboratory studies and are currently in clinical trials.

A correlation between NQO1 catalytic activity and sensitivity of human cancer cell lines and xenografts to GA and 17-AAG was first reported by Kelland et al. [105]. Later it was shown in cell-free systems that purified NQO1 could reduce a series of BQA including 17-AAG to their corresponding hydroquinones [106–108]. These studies also demonstrated that the hydroquinones formed following reduction by NQO1 were relatively stable and could be isolated for analysis but were susceptible to metal-catalyzed oxidation [109]. Polarographic studies showed that the addition of SOD significantly inhibited the oxidation of 17-AAG hydroquinone generated by NQO1 implicating superoxide in the oxidation of 17-AAG hydroquinone [110]. In cell-free studies it was also shown that the hydroquinones formed from BQAs were resistant to direct conjugation by GSH at the 19-position on the ansamycin ring [107]. The ability of cells to generate the hydroquinone would prevent drug inactivation due to GSH conjugation (Fig. 4). The most important feature of the hydroquinone, however, became apparent from cell-free studies that showed that the hydroquinone of 17-AAG was a more potent inhibitor of purified Hsp90 when compared to 17-AAG (quinone) [106]. Computational-based molecular simulation studies with BQAs confirmed these data and showed that the hydroquinones bound with greater affinity to the ATPase active site in Hsp90 when compared with their corresponding quinones [106,108,111]. The greater interaction energies observed with hydroquinone ansamycins can be explained by the stronger and greater number of hydrogen-bonding interactions with key amino acid residues in the ATP-binding domain of Hsp90.

Studies in NQO1 null and NQO1-overexpressing breast and pancreatic cancer isogenic cell lines confirmed earlier observations that NQO1 expression increases sensitivity to 17-AAG [106,110]. HPLC analysis confirmed greater 17-AAG hydroquinone generation in cell lines expressing NQO1 and hydroquinone formation could be inhibited by pretreatment with the NQO1 mechanism-based inhibitor ES936 (5-methoxy-1,2-dimethyl-3-[(4-nitrophenoxy)-methyl]indole-4,7-dione) [106,110]. Intracellular reduction of 17-AAG to the hydroquinone resulted in substantially higher intracellular concentrations of 17-AAG (quinone and hydroquinone) suggesting that the hydroquinone may not diffuse readily

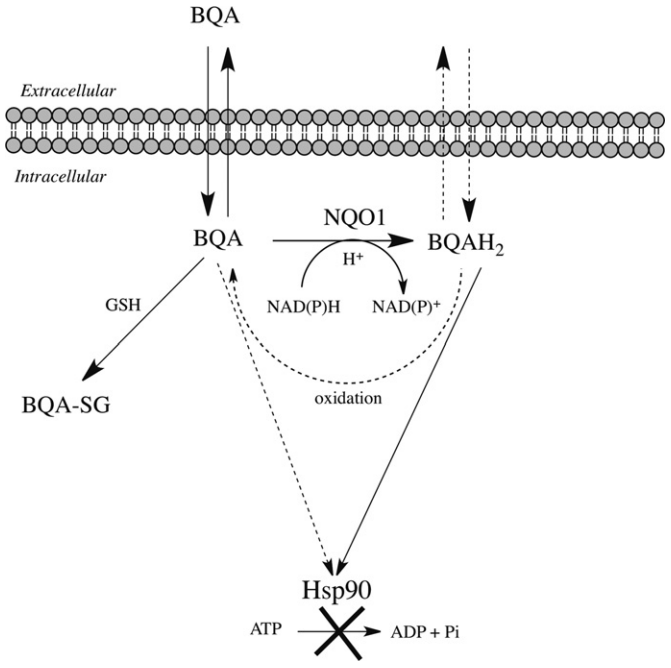

Fig. 4. The role of NQO1 in potentiating the antitumor activity of benzoquinone ansamycins. Solid lines represent major pathways. Dashed lines represent minor pathways. BQA, benzoquinone ansamycin quinone; $BQAH_2$, benzoquinone ansamycin hydroquinone; BQA-GS, benzoquinone ansamycin quinone-glutathione conjugate; Hsp90, heatshock 90 protein.

across cell membranes (Fig. 4). Correspondingly, biomarkers of Hsp90 inhibition were more pronounced in cells expressing NQO1 compared to NQO1 null cells [106,110]. Moreover, human glioblastoma and melanoma cell lines made resistant to 17-AAG by continuous low dose exposure had markedly decreased NQO1 activity and protein [112]. Interestingly, genetic analysis revealed that the resistant glioblastomas had acquired mutations in NQO1 and now expressed the NQO1 null (NQO1*2*2) genotype [112]. Taken together these data strongly implicate a role for NQO1 and reduction to the hydroquinone in the cytotoxicity of BQAs.

IPI504 (Retaspimycin) is the hydroquinone of 17-AAG and was developed as a more water-soluble alternative to 17-AAG. Interestingly, during the same time period it was demonstrated by our own work that the hydroquinone of 17AAG was the active Hsp90 inhibitor and was markedly more potent that parent quinone [106]. While studies using human tumor isogenic cell lines from colon, breast and pancreas which express a range of NQO1 protein levels showed a positive correlation between NQO1 activity and sensitivity to 17-AAG, a study using the 17-AAG prodrug IPI504 did not reach the same conclusion [113]. In experiments where cell lines were treated with IPI504 for 3 consecutive days no significant correlation was observed between NQO1 activity and growth inhibition induced by IPI504. These conclusions were puzzling since the most sensitive cell lines had the highest levels of NQO1 activity and cell lines genotyped as homozygous for the NQO1*2 polymorphism (MDA468 and MDA231) were the most resistant to IPI504 [113]. The lack of sensitivity to IPI504 in cells homozygous for NQO1*2 polymorphism is consistent with data obtained with 17-AAG [105,106,110,114].

Despite studies which show resistance to BQAs in cell lines homozygous for the NQO1*2 polymorphism, there are no published studies at this time that have examined whether clinical response to BQAs is associated with the NQO1*2 genotype. A single study has examined the effect of the NQO1*2 allele on 17-AAG distribution and toxicity in a small number of patients in a phase 1

clinical trial. In this study no correlation was observed between the NQO1*2 allele and 17-AAG distribution and toxicity [115]. However, it was not clear if any of the participants in this study were homozygous for the NQO1*2 polymorphism.

1.5. NQO1 in the clinic

The use of NQO1 as a predictive biomarker for sensitivity to quinone antitumor drugs is compelling. NQO1 activity and protein expression can be easily measured in biopsied tumor samples but the results of a single biopsy may not be an accurate predictor of NQO1 activity over the course of therapy. NQO1 protein expression can be rapidly induced by a host of dietary components, xenobiotics and environmental factors. Therefore, the predictive value of immunohistochemical staining for NQO1 is limited since NQO1 levels may vary considerably over time. An alternative test for predicting NQO1 expression in tumors would involve genotyping patients for the NQO1*2 polymorphism. This simple genetic test may be a more practical measure of the influence of NQO1 on quinone drug activation since this test would identify NQO1-null individuals. Complicating the association between the NQO1*2 polymorphism and response to chemotherapy are studies in women with breast cancer that have demonstrated an association of the NQO1*2 polymorphism with decreased survival in patents receiving anthracycline therapy [116,117], however, in these studies the role of NQO1 in survival is believed to be linked to its role in p53 stabilization and or modulation of TNF-alpha and not through drug activation [116].

Given the substantial amount of preclinical data clearly implicating NQO1 in the bioactivation of quinones such as β-lapachone and 17-AAG it would be predicted that NQO1 null individuals would respond less favorably or may have increased toxicities due to their inability to efficiently activate these drugs in tumor cells. However, to date there are no published papers that have examined whether the NQO1*2 polymorphism correlates with clinical outcomes for patients in clinical trials with β-lapachone and 17-AAG. Hopefully in the future as β-lapachone and the 17-AAG progress through clinical trials with larger numbers of patients the question of whether NQO1 plays a clinically significant role in the antitumor activity of these drugs will be addressed.

2. Summary

NQO1 is important in the bioreductive activation of a number of different types of antitumor quinones. The role of NQO1 in the bioreductive activation of mitomycin C remains controversial but pre-clinical data strongly suggests a role for NQO1 in the activation of β-lapachone and the benzoquinone ansamycin class of Hsp90 inhibitors. Currently, there is little clinical data to reinforce the biological relevance of NQO1 in either sensitivity or resistance to antitumor quinones but the availability of simple assays for the determination of a relatively common null polymorphism in NQO1 should facilitate clinical testing of this hypothesis in the future.

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