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Functions and distribution of NQO1 in human bone marrow: Potential clues to benzene toxicity

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Abstract

NADPH:quinone oxidoreductase 1 (NQO1) may perform multiple functions within the cell. It is known to detoxify benzene-derived quinones and generate antioxidant forms of ubiquinone and Vitamin E. Recently suggested roles for NQO1 which may have relevance for mechanisms underlying benzene toxicity include modulation of cellular redox balance, direct scavenging of superoxide, stabilization of p53 and stabilization of microtubules. The NQO1*2 polymorphism is a single nucleotide polymorphism, a C to T change at position 609 of the NQO1 cDNA coding for a proline to serine change at position 187 of the amino acid structure of the protein. The mutant NQO1*2 protein is rapidly degraded by the ubiquitin proteasomal system resulting in a lack of NQO1 protein in individuals carrying the NQO1*2/*2 genotype. The NQO1*2 polymorphism predisposes to benzene toxicity and to various forms of leukemias. NQO1-knockout animals demonstrate myeloid hyperplasia and increased benzene-induced hematotoxicity. NQO1 is not present in freshly isolated human bone marrow hematopoietic cells but can be induced by benzene metabolites. Increases in NQO1 were not observed in NQO1*2/*2 hematopoietic cells, presumably because of the instability of the NQO1*2 protein, suggesting that cells with this genotype would not benefit from any protective effects of NQO1. NQO1 is present in human bone marrow stroma and particularly in endothelial cells. Studies of the functions and distribution of NQO1 in human bone marrow may provide clues to mechanisms underlying benzene toxicity.

Keywords: Benzene; Quinones; NADPH:quinone oxidoreductase 1 (NQO1); Genetic polymorphisms; Bone marrow; Toxicity; Leukemia

1. Background

NADPH:quinone oxidoreductase 1 (NQO1); DT-diaphorase is a flavin-containing quinone reductase with a broad substrate specificity [1]. Its preferred substrates are quinones and the mechanism of reduction using quinones as substrates is considered to be obligate two electron reduction functioning via a hydride transfer mechanism [2–4]. NQO1 functions with equal facility using either NADH or NADPH as electron donors [5] and the gene is located at 16q22.1 in the human chromosome [6–8]. NQO1 is highly inducible by many stimuli including electrophilic metabolites and oxidative stress [9,10] and may be thought of as a stress reponse. Induction is considered to occur via

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both ARE and XRE elements in the NQO1 promoter [11,12] and is also under the control of the Nrf2/Keap1 system [13,14]. The enzyme is mainly cytosolic although small mitochondrial and microsomal pools have been identified in rat liver [1,15,16]. A small nuclear pool of NQO1 has been characterized in human tumor cells [17] and in humans, NQO1 activity is found mainly in epithelial and endothelial tissues [18]. We were not able to detect significant quantities of NQO1 in human liver samples [18] although NQO1 is expressed at high levels in both mouse and rat liver [19,20] indicating a significant species difference in expression. The crystal structure of the human form of NQO1 has been described [21] and has been helpful in the investigation of potential substrates and inhibitors. Structural studies indicate a highly plastic active site which can accommodate a wide variety of substrates [4,22].

NQO1 has long been generalized as a detoxification step in quinone metabolism because it leads to the formation of a more water soluble and, therefore, more easily excreted hydroquinone metabolite [23]. However, care should be taken in generalizing the effects of NQO1 across such a broad chemical class as quinones since two electron reduction may lead to a more reactive hydroquinone metabolite [12]. This has been shown to be the case with highly redox active hydroquinones which can autoxidize to generate aggressive oxygen species or some hydroquinones may rearrange to generate reactive alkylating agents [24,25]. The latter pathway is particularly prevalent with antitumor quinones and since NQO1 is expressed at high levels throughout many human tumor cells [18,26], the design of compounds efficiently bioactivated by NQO1 has been a fruitful area for anticancer drug development [12,27]. With respect to benzene-derived quinones, however, there is convincing evidence that NQO1 acts as a detoxification system (see below).

2. The NQO1*2 polymorphism: genotype–phenotype relationships

Two single nucleotide polymorphisms (SNPs) in NQO1 have been characterized, the NQO1*2 polymorphism and the NQO1*3 polymorphism [28,29]. The allele frequency of the NQO1*3 polymorphism is low [30] and the phenotypic consequences of the NQO1*3 polymorphism are variable according to substrate [28]. Nebert et al. [31] have identified an additional 22 variants in NQO1 from a screen of a SNP database but the frequency of these genetic variants in the population and their significance for phenotype is presently unknown. This review will focus on the NQO1*2 polymorphism.

The NQO1*2 polymorphism [32–34] is a single nucleotide change at position 609 of the NQO1 cDNA coding for a proline to serine change at position 187 in the amino acid structure of the protein (Fig. 1). The polymorphism has significant phenotypic consequences [35] and results in a lack of NQO1*2 protein in tissue samples from individuals carrying the homozygous NQO1*2 polymorphism. Comparisons between

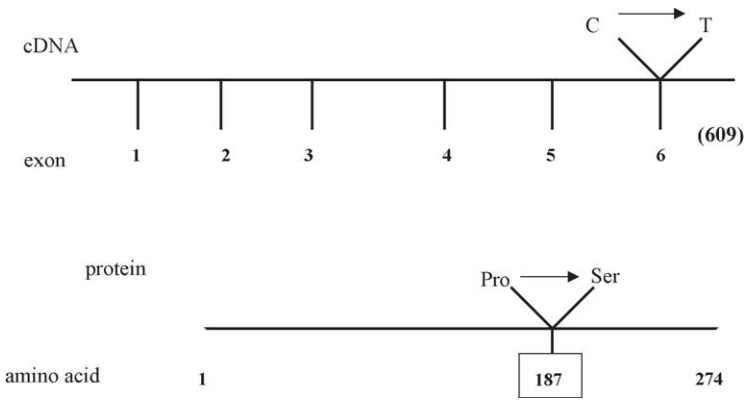

Fig. 1. NQO1 gene and the location of the NQO1*2 polymorphism.

the NQO1*1/*1 genotype and the NQO1*2/*2 genotype demonstrated similar levels of mRNA and similar mRNA half lives in cells and similar rates of transcription and translation in a coupled cell-free system [36]. These data suggested that the defect with respect to NQO1 expression was at the level of the protein. Indeed, the mutant NQO1*2 protein was found to be degraded rapidly by the ubiquitin proteasomal system with a half life of approximately 1.5 h [36]. The wild type protein is stable in cells for periods longer than 18 h so the homozygous NQO1*2 polymorphism confers a major phenotypic change. Individuals heterozygous for the NQO1*2 polymorphism (i.e. NQO1*1/*2 genotype) have amounts of protein and activity levels intermediate between NQO1*1/*1 (large amounts of stable protein) and NQO1*2/*2 (no protein detected in tissues, trace levels of protein detected in cultured cell systems). Since the NQO1 gene is highly inducible, however, there can be a considerable range of NQO1 levels in both wild type and heterozygous individuals [35].

The mechanisms underlying the instability of the mutant NQO1*2 protein are unclear. The proline to serine mutation is in a beta turn and may adversely affect the structure of the protein tagging it for degradation by the ubiquitin proteasomal system. We have also shown that the NQO1*2 protein, unlike the NQO1*1 protein, does not associate with the protein chaperones Hsp70 and Hsp40 in a multi-protein complex [37]. Protein chaperones serve as catalysts which help newly synthesized proteins fold correctly and then they dissociate from the protein. Mutation of an Hsp70 binding site near the N terminus of the NQO1 protein prevents association of newly synthesized NQO1*1 protein with the chaperone complex [37]. In agreement with the “protein catalyst” function of the chaperones, Hsp70 does not interact with fully folded recombinant NQO1 but only with newly synthesized forms of the protein [37]. Thus, lack of interaction of the NQO1*2 protein with heat shock proteins may lead to aberrant folding and accelerated degradation through the ubiquitin proteasomal system.

The prevalence of the NQO1*2/*2 genotype (Table 1) varies in different ethnic groups but is as high as 22% in Chinese populations [34] and recent work has reported an even higher prevalence of the homozygous NQO1*2/*2 genotype in ethnic Hmong populations living in the US of 34% [38]. Interestingly, the prevalence of the NQO1*2/*2 genotype in Caucasians is in agreement with an early phenotype study published many years prior to characterization of the NQO1*2 polymorphism. This study reported that 4% of samples from a British population lacked NQO1 activity [39].

Table 1
Percentage of individuals in different populations with the NQO1*2/*2 genotype

Population	NQO1*2/*2 (%)
Hmong	34.0
Chinese	22.4
Korean	18.8
Native American	17.9
Mexican Hispanic	15.5
Japanese	12.2
African American	5.2
Non-hispanic white	4.4

Data are taken from [80,38,34].

3. The NQO1*2 polymorphism: potential role in benzene toxicity and susceptibility to leukemias

Benzene metabolism has been well characterized [40,41] and a critical review of specific metabolites in benzene toxicity has been published [42]. One potential mechanism of benzene toxicity involves metabolism by cytochrome P4502E1 (CYP2E1) to phenolic metabolites which accumulate in bone marrow [43]. After autoxidation of these metabolites such as hydroquinone or myeloperoxidase (MPO) catalayzed oxidation, reactive quinones are generated which may lead to cellular damage and/or toxicity (Fig. 2). Experiments in cytochrome P4502E1 knockout animals have indicated the importance of CYP2E1 to benzene metabolism and toxicity [44] while studies in cell culture systems have indicated the importance of NQO1 in benzene-derived quinone detoxification in bone marrow stroma [45,46]. Fig. 2 also demonstrates the potential impact of the NQO1*2 polymorphism.

There are three major pieces of evidence linking the NQO1*2 polymorphism to benzene toxicity and hematopoietic disorders.

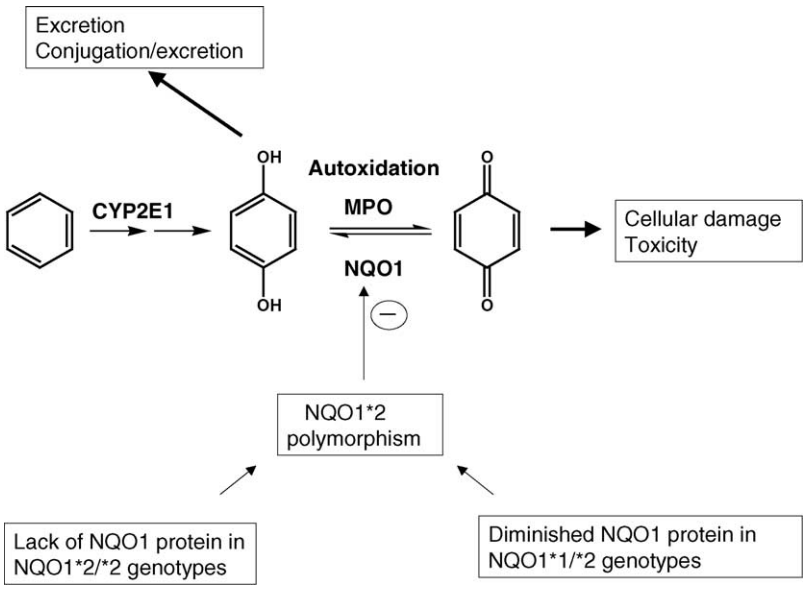

Fig. 2. A proposed mechanism of bioactivation of benzene leading to the generation of reactive quinone metabolites in bone marrow. The scheme shows the importance of cytochrome P4502E1 (CYP2E1) and NQO1 in benzene metabolism. Any quinone generated in the bone marrow as a result of autoxidation or myeloperoxidase (MPO) catalyzed oxidation of hydroquinone can be reduced back to hydroquinone by NQO1. Hydroquinone can be more readily conjugated and/or excreted. There is little NQO1 in hematopoietic cells in bone marrow although stromal cells, particularly endothelial cells, contain substantial levels of NQO1 (see text). The figure also shows the potential impact of the NQO1*2 polymorphism which leads to either a lack (NQO1*2/*2) or diminished (NQO1*1/*2) levels of NQO1 protein (see text).

*3.1. Increased risk of benzene induced hematotoxicity associated with the NQO1*2 polymorphism*

The interest in the relationship of the NQO1*2 polymorphism to benzene toxicity began with a case control study performed as a part of the NCI-Shanghai study [47]. This work demonstrated that individuals with the NQO1*2/*2 genotype who were occupationally exposed to benzene were at a 2.4-fold increased risk of benzene-induced hematotoxicity relative to matched controls. Interestingly, the NQO1*2/*2 genotype when coupled with a rapid cytochrome P4502E1 metabolizer phenotype (as indicated by chlorzoxazone hydroxylation) led to a 7.6-fold increased risk. These results demonstrated the importance of cytochrome P4502E1 and NQO1 in the metabolic events leading to expression of benzene toxicity in a human population.

*3.2. Increased risk of leukemia associated with the NQO1*2 polymorphism*

There are now six epidemiological studies associating the NQO1*2 polymorphism with an increased risk of leukemia [34]. These include increased risks of therapy related leukemia [48], therapy related leukemia/myelodysplastic syndrome [49], pediatric leukemias (particularly with MLL gene rearrangements) [50,51], childhood acute lymphoblastic leukemia [52] and adult de-novo leukemia [53]. Two studies have found no association of the NQO1*2 polymorphism with de-novo or therapy related leukemia [54,55].

3.3. Studies in the NQO1 knockout mouse

The third critical piece of evidence linking the NQO1*2 polymorphism to benzene toxicity and leukemia have been studies in the NQO1 knockout

mouse. Long et al. have described myeloid hyperplasia in NQO1-knockout animals [56] and this finding represents important evidence supporting a role for NQO1 in hematopoietic diseases. Importantly, Bauer et al. also demonstrated that benzene exposure of NQO1-knockout animals resulted in increased hematotoxicity relative to wild type controls [57].

4. Can studies on NQO1 yield clues to mechanisms underlying benzene toxicity?

Given the associations of the NQO1*2 polymorphism with benzene toxicity in exposed workers, the association of the polymorphism with various forms of leukemia, the appearance of myeloid hyperplasia in NQO1-knockout animals and increased hematotoxicity of benzene in NQO1-knockout animals, we asked the question whether we could use studies of NQO1 to yield clues to mechanisms underlying benzene toxicity. We pursued this question by investigating (A) the functions of NQO1 and (B) the sites of expression of NQO1 in human bone marrow.

4.1. Functions of NQO1

It appears that NQO1 may have multiple functions within a cell [58]. Potential functions that have been described for NQO1 are summarized below.

4.1.1. Metabolism of xenobiotic quinones to hydroquinones

The traditional metabolic event associated with NQO1 is two electron reduction of a quinone to a hydroquinone. As stated above, the hydroquinone is more readily excreted, two electron reduction bypasses semiquinone and subsequent oxygen radical generation and also removes an electrophilic quinone species from a biological system [59,60]. NQO1 has been shown to be a detoxification system for benzoquinones in bone marrow cells and a high myeloperoxidase/NQO1 ratio has been suggested to be a predisposing factor for toxicity in such cells [46,61]. Importantly, the human bone marrow CD34+ progenitor cell has been shown to have a high MPO/NQO1 ratio [61–64].

4.1.2. Metabolism of endogenous quinones to hydroquinones resulting in antioxidant effects

This function of NQO1 is an extension of (1) but applied to endogenous quinones. NQO1 has been shown to catalyze reduction of ubiquinone to ubiquinol [65,66] and Vitamin E quinone to Vitamin E quinol [67]. Both quinones are devoid of antioxidant capability before reduction and the hydroquinone forms are potent antioxidants. This suggests that NQO1 can function in an indirect manner as an antioxidant enzyme. Since phenolic metabolites of benzene are known to autoxidize generating oxygen radicals, this mechanism may be of relevance to benzene toxicity.

4.1.3. Redox balance

NQO1 utilizes both NADH and NADPH and is contained in some cells at high levels. This has led to the suggestion that it may assist in controlling the redox balance in the cell by modulating reduced/oxidized pyridine nucleotide ratios and, thus, affect cellular signaling [56,68]. Inhibition of NQO1 may lead to loss of NAD+ which could affect polyADP ribose polymerase function and the function of other proteins requiring NAD+ [68]. It has also been reported that NAD+ plays an important role in control of gene expression in yeast [68] which would support a proposed regulatory role for the pyridine nucleotide redox balance.

4.1.4. Direct oxygen radical scavenging

In recent work, we have demonstrated that NQO1 can function as a direct superoxide scavenging enzyme [69]. The interaction of NQO1 with superoxide is dependent on pyridine nucleotide cofactor and can be inhibited by mechanism based inhibitors of NQO1 demonstrating the requirement for catalytic activity of NQO1. The proposed mechanism is dependent on generation of the flavin hydroquinone and subsequent interaction of superoxide with the reduced flavin [69]. The overall process represents a superoxide reduction and the end product of the reaction is hydrogen peroxide.

Superoxide dismutase interacts with superoxide with a rate constant of $1.7 \times 10^9\,M^{-1}\,s^{-1}$ while the rate constant for interaction of NQO1 with superoxide is at least four orders of magnitude lower [69]. Thus, NQO1 would not be expected to compete with SOD for superoxide on an equimolar basis of protein. However, in cell cytosols where NQO1 is expressed at very

high levels we have shown that NQO1 can contribute to the superoxide scavenging ability of the cell [69]. This could explain why NQO1 is induced to very high levels by oxidative stress [9]. A direct oxygen radical scavenging effect may help to protect against oxidative stress generated as a result of exposure to benzene metabolites.

4.1.5. Interaction with and stabilization of p53

In a series of papers by Asher et al., NQO1 has been reported to stabilize p53 [68,70,71] and the mechanism proposed was a redox mechanism (see above). We have also demonstrated that NQO1 interacts with p53 in a protein-protein interaction [72] and it is possible that such an interaction may also have functional consequences for p53 stability and function. There are many proteins that are known to interact with p53 and the consequences of such an interaction may well be cell type and stress dependent. Stabilization of p53 by NQO1, whether mediated via a redox mechanism or via a protein-protein interaction, represents an intriguing possibility that could help explain the long-recognized chemoprotective effects of NQO1 in many different systems.

4.1.6. Stabilization of microtubules

In 2004, Wignall et al. [73] screened over 1500 compounds in a xenopus system to identify proteins involved in microtubule stabilization. The most potent compound identified with respect to inhibition of microtubule stability was also a potent NQO1 inhibitor. Experiments using immunodepletion and other NQO1 inhibitors identified NQO1 as a factor leading to the stabilization of microtubules. Since inefficient and/or incomplete microtubule assembly may result in problems in chromosome segregation, it is conceivable that this effect may have importance for the adverse effects of benzene in hematopoietic cells. It would also be interesting to examine microtubule stability as a function of exposure to benzene metabolites in the presence or absence of the NQO1*2 polymorphism.

4.2. Expression of NQO1 in human bone marrow

4.2.1. Human bone marrow mononuclear cells and CD34+ progenitor cells

We were unable to demonstrate the presence of NQO1 using either activity assays or immunoblotting [64] in freshly isolated human bone marrow mononuclear cells (HBMMC) or human CD34+ progenitor cells (HPC). However, after either HBMMC or HPC cells were incubated with phenolic metabolites of benzene, NQO1 was induced as detected by increased activity and protein levels [64]. Interestingly, NQO1 activity was not increased after exposure of HBMMC homozygous for the NQO1*2 mutation to hydroquinone. Increased NQO1 activity could be observed in cells genotyped as NQO1*1/*1 and a smaller increase was observed in cells genotyped as NQO1*1/*2. The lack of increased NQO1 in NQO1*2/*2 cells after exposure to hydroquinone probably reflected the instability of the mutant NQO1*2 protein (see above).

The implication of these studies is that exposure of HBMMC and HPC that are homozygous for the NQO1*2 polymorphism to phenolic metabolites of benzene will not result in increased NQO1 levels. Thus, individuals carrying the homozygous NQO1*2 polymorphism would not benefit from any protective effects of NQO1 after exposure to benzene metabolites (Fig. 3). This may be of importance to benzene toxicity and may explain the increased susceptibility of individuals carrying the NQO1*2 polymorphism to benzene-induced hematopoietic toxicity.

4.2.2. Human bone marrow stroma

We have previously demonstrated that human bone marrow-derived fibroblastoid cells contain appreciable NQO1 [45]. More recently, we have used immunohistochemistry and bone marrow core biopsies to investigate further the location of NQO1 in human bone marrow. Our data confirmed a lack of NQO1 in hematopoietic cells within the marrow but demonstrated the presence of NQO1 in endothelial cells lining large blood vessels and sinusoids in the marrow [74]. NQO1 could also be observed in adipocytes in human bone marrow. The significance of the presence of NQO1 in bone marrow stroma, and particularly in endothelial cells, requires further investigation. The endothelium is involved in progenitor cell adhesion, proliferation, differentiation and controls migration of progenitors across the endothelial cell barrier [75–78]. These effects may have relevance for the hematotoxic and leukemogenic effects of benzene. We have recently shown that benzene metabolites have the capability to modulate production of cytokines from human bone marrow endothelial cells [79]. The function of endothelial cells with

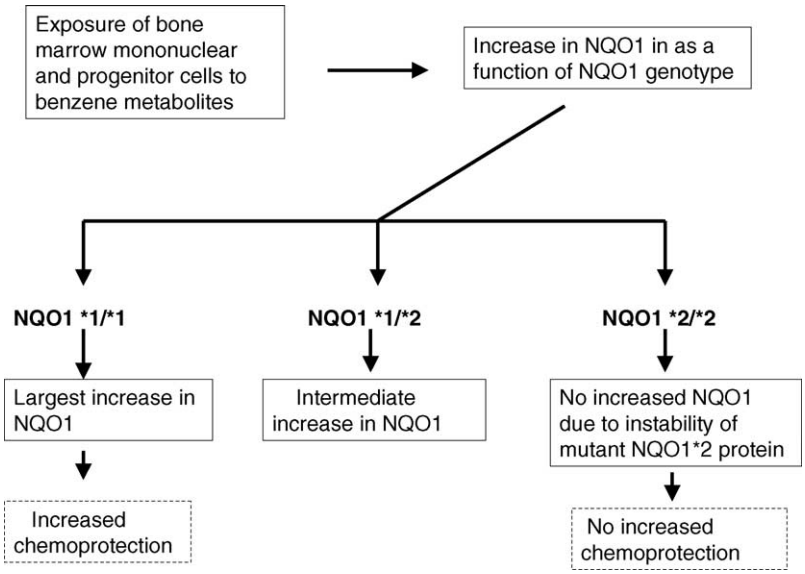

Fig. 3. Induction of NQO1 in hematopoietic cells by phenolic metabolites of benzene. This scheme provides a hypothesis for the susceptibility of bone marrow cells with the NQO1*2/*2 genotype to benzene metabolites. Benzene metabolites induce NQO1 in hematopoietic cells with wild type and heterozygous genotypes while in cells carrying the homozygous NQO1*2 variant, no induction of NQO1 could be detected, presumably because of the instability of the NQO1*2 mutant protein. This suggests that cells with the NQO1*2/*2 genotype would not benefit from any chemoprotective effects that might result from induced levels of NQO1 after exposure to benzene metabolites.

diminished NQO1 activity as a result of the NQO1*2 polymorphism after exposure to benzene metabolites is worthy of future investigation.

5. Summary

The NQO1*2 polymorphism predisposes to benzene toxicity and to various forms of leukemia in humans. NQO1-knockout animals demonstrate myeloid hyperplasia. This suggests NQO1 may be an important susceptibility gene for benzene toxicity and hematopoietic disease. Studies of the functions and distribution of NQO1 in human bone marrow have yielded clues to mechanisms underlying benzene toxicity. NQO1 may have multiple functions in the cell and newly discovered roles with potential relevance for benzene toxicity include direct superoxide scavenging, stabilization of p53 and stabilization of microtubules. Distribution studies demonstrate high levels of NQO1 in endothelial cells in human bone marrow while hematopoietic cells are devoid of NQO1. However, NQO1 can be induced in hematopoietic cells and progenitors by benzene metabolites but this is dependent on genotype. Because of the instability of the mutant NQO1*2 protein, NQO1 activity is not increased after exposure of NQO1*2/*2 cells to benzene metabolites suggesting that cells carrying the NQO1*2 polymorphism will not benefit from the chemoprotective functions of NQO1. These observations suggest that studies of NQO1 are relevant to the elucidation of mechanisms underlying benzene toxicity.

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