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Benzene toxicity: The role of the susceptibility factor NQO1 in bone marrow endothelial cell signaling and function

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Abstract

The homozygous NQO1*2 polymorphism results in a null NQO1 phenotype and is a susceptibility factor for occupational benzene poisoning. NQO1 plays an important role in detoxification of benzene-derived quinones but plays a role in numerous other non-metabolic cellular functions. NQO1 is expressed in endothelial cells of bone marrow which form the vascular stem cell niche important in stem cell homing and mobilization. We therefore employed a transformed human bone marrow endothelial cell (HBMEC) line to define the effects of compromising NQO1 on endothelial function. Either inhibition or knockdown of NQO1 led to decreased expression of the adhesion molecules E-selectin, VCAM-1 and ICAM-1 and decreased functional adhesion of CD34+ progenitor cells after TNF α stimulation. Suicide inhibition or knockdown of NQO1 decreased NF κ B p105 precursor and NF κ B p50 subunit levels as well as leading to decreased nuclear levels of NF κ B phospho-p65. An additional function of endothelial cells is tube formation and angiogenesis which was inhibited by the benzene metabolite hydroquinone suggesting that endothelial function may be affected at multiple levels after exposure of NQO1*2 polymorphic individuals to benzene. These data demonstrate that NQO1 plays an upstream role in NF κ B signaling and adhesion molecule expression in HBMEC and that NQO1 has important regulatory effects in its own right in addition to being a marker for Nrf-2 activation. Metabolic susceptibility factors such as NQO1 have roles in addition to detoxification of reactive intermediates and interrogation of these novel roles can inform both mechanisms of toxicity and human risk assessment.

Keywords

Benzene; NAD(P)H:quinone oxidoreductase 1 (NQO1); adhesion molecule; NF κ B; bone marrow endothelium; vascular stem cell niche

1. NAD(P)H:Quinone oxidoreductase 1 (NQO1) and Benzene Toxicity

A null polymorphism in NQO1, the NQO1*2 polymorphism, has been associated with toxicity after occupational exposure of workers to benzene [1–4]. This effect has been

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considered a result of a lack of metabolic detoxification of reactive benzene-derived quinones in individuals deficient in NQO1. This interpretation is consistent with *in-vitro* data demonstrating that NQO1 is one of the metabolic determinants of the cell-specific toxicity of benzene metabolites in bone marrow cell populations [5–8]. However, it has become increasingly evident that NQO1 has multiple biological functions and the consequences of the NQO1*2 polymorphism may not be restricted to metabolism [9–11]. NQO1 has been associated with control of reduced/oxidized pyridine nucleotide ratios [11], superoxide scavenging [12], interaction with and modulation of proteasomal processing of p53 [13–15] and other proteins [16,17] and microtubule stabilization [18]. The concept that proteins associated with metabolism play diverse roles in cellular function is not new and examples include regulation of JNK signaling and cell proliferation by glutathione-S-transferases [19] and regulation of hematopoietic stem cell cycling and the balance between stem cell quiescence/proliferation by the Ah receptor [20–22]. The latter effect may be particularly relevant to mechanisms underlying benzene hematopoietic toxicity.

1.1 Additional roles of NQO1 influencing hematopoiesis ?

In addition to the association of increased benzene toxicity with compromised NQO1 status, the NQO1*2 polymorphism has also been found to be a susceptibility factor for *de-novo* leukemias [23–25] and for therapy-induced leukemias [26–28]. Further, unchallenged NQO1 knockout animals demonstrate myeloid hyperplasia [29] and disruption of the NQO1 gene in mice leads to radiation induced myeloproliferative disease [30]. These findings have led us to examine whether NQO1 might be influencing hematopoiesis directly in addition to its metabolic detoxification role.

2. NQO1 and bone marrow endothelium

In archived human bone marrow biopsies, NQO1 was found to be expressed at high levels in bone marrow endothelium [31] which is of potential functional significance. A single layer of bone marrow sinusoidal endothelial cells forms the stem cell vascular niche which is important in the trafficking of stem cells between bone marrow and peripheral compartments [32–34]. In addition, bone marrow endothelial cells have recently been shown to be essential for the self renewal and repopulation of stem cells [35]. Important functions of endothelial cells include adhesion molecule expression which is critical in stem cell homing to and mobilization from bone marrow [32–34] and capillary tube formation which is essential for angiogenesis [36,37]. Recently, recovery after myelosuppression, which occurs after benzene exposure, has been shown to depend on endothelial cell angiogenesis [38]. If a lack of NQO1 as a consequence of the NQO1*2 polymorphism resulted in compromised bone marrow endothelial cell function, it is conceivable given the key role of endothelium in both adhesion and angiogenesis that this may result in impaired hematopoiesis. To explore the question of whether a lack of NQO1 alters bone marrow endothelial cell function we have employed a transformed human bone marrow endothelial cell line which has been used to investigate mechanisms underlying adhesion of bone marrow progenitor cells to endothelium [39]. Multiple adhesion molecules and chemokines are likely important players in the adhesion process but in the transformed human bone marrow endothelial cell line employed in our studies, E-selectin, VCAM-1 and ICAM-1 have been demonstrated to play key roles. E-Selectin is important in the initial rolling behavior of attaching cells while VCAM-1 and ICAM-1 contribute to firm adhesion [39].

2.1 Does compromising NQO1 in human bone marrow endothelial cells result in modulation of adhesion molecule expression and functional adhesion of CD34+ progenitor cells ?

Using the transformed human bone marrow endothelial cell line (HBMEC), we first examined modulation of gene expression as a result of inhibition of NQO1 using mechanism-based (suicide) inhibitors of NQO1. Interestingly, data indicated that VCAM-1 was one of the genes transcriptionally down-regulated as a result of NQO1 inhibition and we then confirmed that either pharmacological inhibition or siRNA knock down of NQO1 led to decreased expression of VCAM-1 at the protein level in HBMEC [40]. TNF- α has been shown to markedly induce VCAM-1, E-selectin and ICAM-1 expression in HBMEC [39] and has been found to be critical to hematopoietic stem cell function and homing [41]. We therefore examined the effects of compromising NQO1 either pharmacologically or genetically on adhesion molecule expression in HBMEC after TNF- α stimulation. TNF- α led to increased expression of E-selectin, VCAM-1 and ICAM-1 in HBMEC and either pre-treatment with inhibitors of NQO1 or anti-NQO1 siRNA resulted in a marked reduction of adhesion molecule expression in HBMEC [42].

The functional correlate of decreased adhesion molecule expression is decreased adhesion of stem and progenitor cells to HBMEC. To explore this question we employed a flow chamber system to examine altered attachment of CD34+ KG1a cells to HBMEC under conditions of constant shear stress. Our data demonstrated approximately 60–70% decreased adhesion of CD34+ progenitor cells to HBMEC after inhibition or knockdown of NQO1 [42].

3. Signaling events modulated by compromising NQO1 in HBMEC. The potential importance of the NF κ B system

The NF κ B system is an important regulatory pathway involved in control of TNF- α stimulated adhesion molecule expression. I κ B α is an inhibitor of NF κ B activation and since previous work has demonstrated that NQO1 deficiency can regulate TNF- α signaling by inhibiting I κ B α phosphorylation [43], we examined levels of I κ B α and its phosphorylated form after treatment of HBMEC with anti-NQO1 siRNA or suicide inhibitors of NQO1. We found no differences in the kinetics of formation and degradation of I κ B α and its phosphorylated form after TNF- α stimulation of HBMEC as a result of compromising NQO1 [44]. Similarly, no differences were observed in levels of nuclear NF κ B p65 in HBMEC as a function of inhibition or knockdown of NQO1. However, we did observe decreased nuclear levels of NF κ B phospho-p65, phospho-c-Jun and ATF2 in HBMEC after compromising NQO1 [42] and all of these signaling systems have been shown to play a role in TNF- α induced gene expression [43,45–47]. These observations provide a potential mechanistic link between altered adhesion in NQO1-compromised endothelial cells and nuclear levels of key transcription factors.

3.1 NQO1 as an upstream regulator of the NF κ B signaling system

Ahn et al.[43] used keratinocytes from NQO1 knockout animals to demonstrate that NQO1 modulated I κ B α kinase activation, I κ B α phosphorylation and I κ B α degradation implicating NQO1 as playing a role in NF κ B signaling. Importantly, NQO1 has very recently been shown to be an upstream regulator of the NF κ B precursor p105 and NF κ B subunit p50 in melanoma [48]. These authors found that NQO1 stabilized BCL3 leading to upregulation of NF κ B p50 which in turn correlated with cell cycle changes mediated by altered cyclin expression and increased proliferation of melanoma cells. NQO1 is mainly a cytosolic enzyme but, consistent with previous work in lung cancer cell lines, Garate et al [48] found significant NQO1 levels in the nucleus of melanoma cells. This suggests that in addition to

its cytosolic functions, NQO1 may play significant roles in the nuclear compartment which may include effects on key transcriptional modulators.

We therefore examined whether inhibition or knockdown of NQO1 might result in a similar effect in HBMEC. As demonstrated in Figure 1, our data is consistent with that of Garate et al [48] and shows decreased p105 and p50 levels as a result of NQO1 inhibition or knockdown in HBMEC implicating NQO1 as an upstream regulator of the NFkB system in HBMEC. We also found significant levels of NQO1 in the nuclear compartment of HBMEC [42] suggesting that nuclear roles for NQO1, potentially at the level of transcription factor stability, need to be explored in more detail.

3.2 NQO1 and adhesion molecule expression in HBMEC

Our current understanding of the role of compromising NQO1 status in impaired endothelial adhesion is shown in Figure 2. Our current working hypothesis is that the impaired adhesive function of the endothelial layer compromises the function of the vascular stem cell niche leading to impaired hematopoiesis. This may provide a common mechanism to explain both the increased incidence of leukemias and the increased myeloid toxicity of benzene exposure in individuals lacking NQO1 as a result of the NQO1*2 polymorphism.

4. Do benzene metabolites affect endothelial cell tube formation in HBMEC?

Another key function of HBMEC is tube formation leading to angiogenesis. Recovery after myelosuppression, which occurs after benzene exposure, has been shown to depend on endothelial cell angiogenesis [38] so we examined HBMEC tube formation after treatment with the benzene metabolite hydroquinone. Hydroquinone treatment of HBMEC led to inhibition of tube formation [49]. Experiments employing gene array analysis indicated that hydroquinone-induced upregulation of chondromodulin 1, an anti angiogenic gene expressed by endothelial cells, was partly responsible for inhibition of tube formation by hydroquinone. Purified chondromodulin 1 reproduced the inhibitory effects of hydroquinone on tube formation and anti-ChM1 siRNA protected against the effects of hydroquinone [49]. The effects of hydroquinone at the level of tube formation in HBMEC are summarized in Fig 3.

5. Summary

Our data indicates the potential importance of NQO1 in bone marrow with respect to both endothelial signaling and endothelial function. Compromising NQO1 in HBMEC led to modulated NFkB transcription, decreased adhesion molecule expression and decreased functional adhesion to stem/progenitor cells [42]. Since adhesion molecules have documented roles in hematopoietic cell homing and mobilization, impairment of the endothelial/vascular stem cell niche may play a role in both the hematopoietic pathology associated with a lack of NQO1 [23–28] and the increased susceptibility of individuals lacking NQO1 to benzene toxicity [1–4].

Another important endothelial function is tube formation and angiogenesis and the benzene metabolite hydroquinone is capable of inhibiting endothelial tube formation [49]. A summary of these two distinct effects, a lack of NQO1 impairing endothelial adhesion and benzene metabolites impairing endothelial tube formation, is summarized in Figure 4. It follows that exposure of NQO1*2 polymorphic individuals to benzene could result in impairment of endothelial function at multiple levels and in future work, whether the bone marrow endothelial cell is a critical target of benzene should be investigated *in-vivo* employing endothelial-specific biomarkers.

Finally, these results could have broader implications for the role of NQO1 in biological systems. NQO1 has been examined in detail but it is often used only as a sentinel marker for activation of Nrf-2 in biological systems. Our observations and previous data of Ahn et al. [43] and Garate et al. [48] suggest that NQO1 has important regulatory effects in its own right in addition to being a marker for Nrf-2 activation. It may be useful to consider these regulatory effects when examining the consequences of modulation of NQO1 in biological systems.

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Figure 1. The effect of compromising NQO1 on the levels of NFκ-B p105 and p50 in HBMEC
Treatment of HBMEC with anti-NQO1 siRNA or the mechanism-based inhibitor ES936 results in decreased levels of p105 and p50. Cells were harvested after treatment with 100nM ES936 for 2h or siRNA for 72h. (siCTL = scrambled siRNA control).

Figure 2.

A proposed mechanism for the role of NQO1 deficiency in the impairment of endothelial progenitor cell adhesion and hematopoiesis.

Figure 3.

The role of ChM-1 in hydroquinone-induced inhibition of tube formation by HBMEC and the protective effect of NQO1.

Figure 4.
The proposed roles of NQO1 deficiency and hydroquinone on bone marrow endothelial cell adhesion molecule expression and angiogenesis.