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Relationships between metabolic and non-metabolic susceptibility factors in benzene toxicity☆

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ABSTRACT

Reactive metabolites formed from benzene include benzene oxide, *trans*,*trans* muconaldehyde, quinones, thiol adducts, phenolic metabolites and oxygen radicals. Susceptibility to the toxic effects of benzene has been suggested to occur partly because of polymorphisms in enzymes involved in benzene metabolism which include cytochrome P450 2E1, epoxide hydrolases, myeloperoxidase, glutathione-S-transferases and quinone reductases. However, susceptibility factors not directly linked to benzene metabolism have also been associated with its toxicity and include p53, proteins involved in DNA repair, genomic stability and expression of cytokines and/or cell adhesion molecules. In this work, we examine potential relationships between metabolic and non-metabolic susceptibility factors using the enzyme NAD(P)H:quinone oxidoreductase (NQO1) as an example. NQO1 may also impact pathways in addition to metabolism of quinones due to protein–protein interactions or other mechanisms related to NQO1 activity. NQO1 has been implicated in stabilizing p53 and in maintaining microtubule integrity. Inhibition or knockdown of NQO1 in bone marrow endothelial cells has been found to lead to deficiencies of E-selectin, ICAM-1 and VCAM-1 adhesion molecule expression after TNFα stimulation. These examples illustrate how the metabolic susceptibility factor NQO1 may influence non-metabolic susceptibility pathways for benzene toxicity.

1. Metabolic factors in susceptibility to benzene toxicity

Benzene induces hematopoietic toxicity and can induce aplastic anemia, myelodysplasia and acute myeloid leukemia after chronic exposure [1,2]. The metabolism of benzene has been investigated extensively and previous reviews have characterized benzene metabolism in a comprehensive manner [3–7]. Consequently, this work is not intended to be a review of benzene metabolism but will focus on metabolic susceptibility factors for benzene toxicity which have been identified in both cell and animal studies and in studies of occupationally exposed populations. Other susceptibility factors not directly linked to metabolism have also been identified in benzene toxicity and relationships between metabolic and non-metabolic susceptibility factors have not been previously considered. We will therefore discuss potential relationships between these two groups of susceptibility factors using the enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1) as an example and highlight recent studies focusing on NQO1 in human bone marrow endothelial cells.

2. Benzene metabolism

Metabolism of benzene is considered necessary for benzene toxicity and the evidence supporting this conclusion has been previously summarized [8–10]. A key finding in animal studies was that knockout of the first step in benzene metabolism mediated by cytochrome P450 2E1 totally abrogated benzene-induced myeloid toxicity and cytotoxicity [11]. Benzene metabolism in liver and *in situ* in bone marrow could both conceivably contribute to benzene-induced myeloid toxicity [10]. A simplified version of benzene metabolism is shown in Fig. 1 where the majority of Phase II metabolic pathways including sulfation and glucuronidation have been omitted. It is important to note however that some Phase II metabolites such as sulfate conjugates have been suggested as carrier forms of phenolic metabolites which are released *in situ* in bone marrow due to a high concentration of sulfatase enzymes and a low content of sulfotransferases [12].

Abbreviations: HBMEC, transformed human bone marrow endothelial cells; MPO, myeloperoxidase; NQO1, NAD(P)H:quinone oxidoreductase 1; GST, glutathione-S-transferase; ChM-I, chondromodulin 1.

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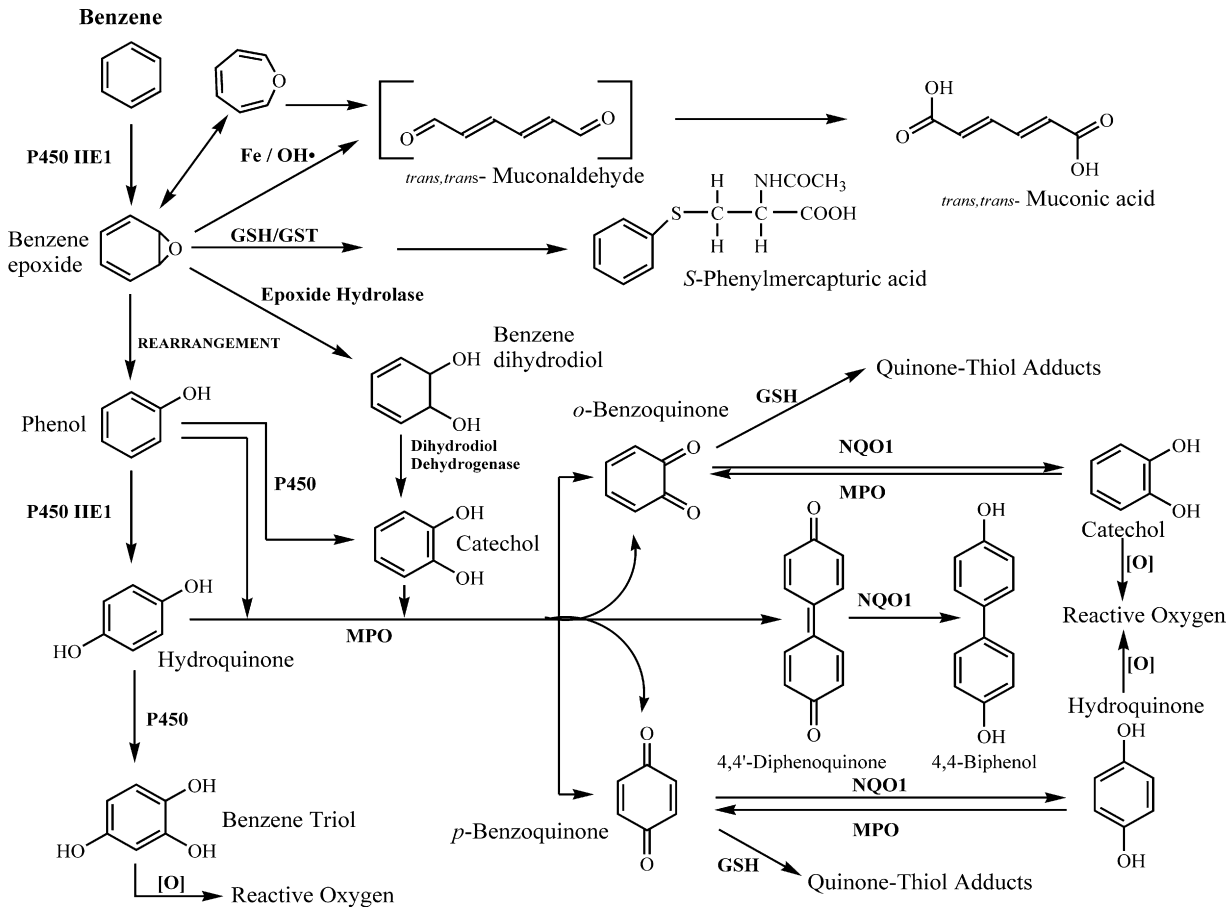

Fig. 1. Benzene metabolic scheme. Most Phase II pathways have been omitted. For potential reactive metabolites, see Table 1. For metabolic susceptibility factors, see Table 2. Adapted from [7,10].

3. Reactive metabolites and metabolic susceptibility factors

Reactive metabolites formed from benzene include benzene epoxide [13–15], *trans*, *trans* muconaldehyde [16–19], phenolic metabolites of benzene [20–22] which can give rise to oxygen radicals upon autoxidation [23,24], reactive quinones and semiquinones formed from polyphenolic metabolites of benzene [25–29] and quinone thiol adducts [30,31] (Table 1). Consequently, the metabolism of benzene is complex and gives rise to a large number of potentially reactive products which have been suggested to be important in benzene toxicity. Metabolic susceptibility factors (Table 2) have been identified in cellular studies, animals and in studies of occupationally exposed human populations. Such susceptibility factors predictably encompass the wide range of benzene metabolic pathways and both phenotypic and genotypic variants of enzymes in these pathways have been investigated in epidemiological studies of benzene toxicity. The first step in benzene metabolism mediated by CYP2E1 represents a key metabolic susceptibility factor [11]. The involvement of other cytochrome P450s in benzene metabolism is also possible and recent work has shown that CYP4F3 was upregulated in peripheral white blood cells in seven patients who had occupational benzene poisoning [32]. In the same study, phenol was found to be capable of inducing CYP4F3 in myeloid cell lines and in human neutrophils [32]. These observations may be significant and could provide a novel metabolic mechanism for benzene-induced myeloid toxicity if CYP4F3 is found to be capable of metabolizing benzene or phenol.

Other metabolic susceptibility factors include epoxide hydrolase which is known to have genotypic variants with a range of activities [33] and glutathione, a key defense system against reactive metabolites [34]. Myeloperoxidase (MPO) can oxidize polyphenolic metabolites of benzene to electrophilic quinones. A promoter polymorphism in MPO (G463A) leads to decreased transcription and decreased enzymatic activity [35] and has

Table 1
Potential reactive metabolites of benzene.

Benzene oxide
Trans,trans muconaldehyde
Quinones
Thiol adducts
Phenolics
Reactive oxygen species

For citations see text.

Table 2
Metabolic susceptibility factors in benzene toxicity.

Factors	Susceptibility pathway
CYP2E1	Rapid metabolizer phenotype and SNP's
CYP4F3	?
MPO	G463A promoter polymorphism leading to decreased transcription
GSH	Enzymes regulating levels
Reactive oxygen	Enzymes regulating levels
EH	Rapid metabolizer genotype, other variants
GST	Null variants in GSTT1 and GSTM1. GSTPi variants with decreased activity
NQO1*2	Heterozygous (decreased activity) and homozygous (null) C609T variants
NQO1*3	Reduced NQO1 activity

For citations see text.