

## Commentary

### Benzene, NQO1, and genetic susceptibility to cancer

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NAD(P)H:quinone oxidoreductase 1 (NQO1; EC 1.6.99.2), originally called DT-diaphorase (1), is an enzyme that has attracted considerable attention because of its ability to detoxify a number of natural and synthetic compounds and, conversely, to activate certain anticancer agents (2, 3). It is also a highly inducible enzyme. Synthetic antioxidants, such as butylated hydroxyanisole, and extracts of cruciferous vegetables, including broccoli, have been shown to be potent inducers of NQO1 (4, 5). This inducibility has led to the suggestion that NQO1 plays an important role in cancer chemoprevention (6).

In 1980, Edwards *et al.* (7) reported that 4% of a British population completely lacked NQO1 activity, but the reasons for and implications of this finding were unclear at the time. In the early 1990s, as part of their studies on the bioactivation of quinone anticancer agents, Ross, Gibson, and their colleagues were characterizing the NQO1 activities of various colon and lung carcinoma cell lines (8). They noticed that two of the lines, the BE colon carcinoma line and the nonsmall cell lung cancer H596 cell line, were different in that they showed no demonstrable NQO1 activity. By using DNA sequencing analysis, they established the presence of a homozygous C to T point mutation at position 609 of the NQO1 cDNA from the BE cell line (8). This mutation conferred a proline-to-serine substitution at position 187 of the NQO1 protein, which they suggested was responsible for the lack of NQO1 activity in BE cells. Sequencing of the coding region of NQO1 from lung H596 cells subsequently showed the presence of the identical homozygous point mutation found in BE cells (9). Thus, the lack of NQO1 activity in certain cell lines and subjects in the Edwards *et al.* study was most likely the result of homozygous inheritance of two mutant alleles at position 609 in the *NQO1* gene. Confirmation of this idea came from the development of a simple PCR-restriction fragment length polymorphism-based method for detecting the 609 C → T polymorphism by Sies and coworkers in Germany (10). NQO1 activity was shown to be absent in three renal carcinoma patients who were homozygous for the mutant allele (11). Recent genotype-phenotype studies *in vivo* have further confirmed that the homozygous C609T change results in a lack of NQO1 enzyme activity and protein (12).

The development of a simple method for detecting the polymorphism meant that it could be examined in human populations. In 1992, together with investigators from the National Cancer Institute and the Chinese Academy of Preventive Medicine, we collected samples of blood from subjects in a case-control study of benzene hematotoxicity in Shanghai, China (13). Benzene is metabolized in the liver to phenol, hydroquinone, and catechol, which then travel to the bone marrow and can be activated by peroxidases to highly toxic quinones (14). NQO1 is capable of maintaining these quinones in their reduced form, thereby detoxifying them. We therefore hypothesized that NQO1 would protect against benzene toxicity and that individuals lacking NQO1 would be at higher risk of benzene poisoning. Analysis of DNA isolated from the subjects in Shanghai by the Ross laboratory (15) revealed that subjects who were homozygous for the 609 C → T polymor-

phism were significantly more likely to be poisoned by benzene (measured as decreased blood cell counts) (odds ratio = 2.6; 95% confidence intervals, 1.1–6.6) and were at elevated risk of contracting benzene-induced leukemia. This work built on a body of evidence from studies *in vitro* by Smart and Zannoni (16) and in animals and cell lines by Trush, Twerdok, and coworkers (17, 18), which suggested that NQO1 protected against benzene toxicity. Our case-control study also revealed the high incidence of the mutant NQO1 allele in the Chinese population with approximately 20% of the population being homozygous mutants, a finding that has been confirmed in other Asian populations (19). The reasons for this high incidence are intriguing, as it is not known what selective pressures are responsible.

A potential problem with our finding of NQO1's protective effect against benzene toxicity in a human epidemiological study was the anomalous observation from the Ross laboratory that freshly isolated human bone marrow cells lacked expression of NQO1 (20). A protective role for NQO1 against benzene-derived quinones in the marrow was difficult to reconcile with this observation. A likely explanation of this apparent anomaly is offered in this issue of the *Proceedings* by Moran, Siegel, and Ross (21), who demonstrate that the benzene metabolite hydroquinone induces high levels of NQO1 activity in bone marrow cells, including CD34<sup>+</sup> progenitor cells, with the wild-type (C/C) genotype. Exposure to noncytotoxic doses of hydroquinone induced intermediate levels of NQO1 activity in heterozygous (C/T) cells, but had no effect in cells with the homozygous mutant (T/T) genotype. Thus, failure to induce functional NQO1 in cells with homozygous mutant alleles may make them susceptible to the toxic effects of benzene metabolites and thereby may explain the increased risk of benzene poisoning in individuals with the (T/T) genotype.

Numerous questions remain, however, about the role NQO1 plays in protecting the body against chemical exposures, the mechanism of its induction by hydroquinone and other chemicals, and the susceptibility of individuals with mutant alleles to various cancers, including leukemia. There is also the interesting biochemical question of why homozygous mutant cells have no NQO1 activity. Ross and coworkers have shown that cells with the homozygous mutant genotype still express significant quantities of NQO1 mRNA but have little or no NQO1 protein (9). Transfection of NQO1 cDNA containing the C609T mutation into *Escherichia coli* and COS-1 cells resulted in expression of mutant NQO1 protein. However, recombinant mutant NQO1 purified from *E. coli* had only 2–4% of the activity of the wild-type enzyme. The reasons for the low activity of the mutant protein are currently under investigation and may be related to its instability.

NQO1 was first called DT-diaphorase after its discovery as a cytosolic diaphorase by Ernster and colleagues in 1958 (2).

Abbreviation: NQO1, NAD(P)H:quinone oxidoreductase 1.

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Quinones, including 1,4-benzoquinone and menadione, were shown to be high-affinity substrates. Subsequently, many xenobiotics, including quinone-epoxides, quinone-imines, naphthoquinones, methylene blue, azo, and nitro compounds, were identified as substrates (3). Interestingly, another proposed toxic metabolite of benzene, *trans,trans*-muconaldehyde (22), is not a substrate for NQO1 and in the paper by Moran, Siegel and Ross (21) in this issue of the *Proceedings*, it is shown that NQO1 induction does not protect against muconaldehyde cytotoxicity. Because NQO1 appears to protect humans against benzene toxicity (15), this suggests that benzoquinones play a more significant role in benzene toxicity than does muconaldehyde. However, the mechanism by which NQO1 protects against benzene toxicity may not be as obvious as it first appears. In 1970, Iyanagi and Yamazaki (23) showed that NQO1 catalyzes the reduction of quinones to hydroquinones without the intermediate formation of the free semiquinone radical. The most obvious hypothesis for the protection afforded by NQO1 against benzene toxicity is therefore that NQO1 maintains benzoquinones in their reduced hydroquinone form and prevents the formation of covalently binding species such as quinones and semiquinones. We have recently investigated this hypothesis by constructing an HL60 myeloid cell subline transfected with the NQO1 gene that had a 34-fold higher activity of NQO1 than the control HL60 cells (24). To our surprise, this high level of NQO1 expression provided only a modest protection against hydroquinone-induced cell death. Further, similar levels of protein binding from [<sup>14</sup>C]-hydroquinone were observed in the control HL60 cells and NQO1-transfected subline (24), which argues against the idea that NQO1 is preventing the arylation of cellular macromolecules in the marrow and is thereby a reducing benzene toxicity. High NQO1 expression in the subline did, however, dramatically decrease the level of a class of as yet unidentified low-mobility DNA adducts that appear to be derived from reactive byproducts of benzene metabolites in the cells (24). It did not, however, alter the level of hydroquinone-specific DNA adducts resolved as described by Lévy and Bodell (25). These findings tend to support the notion that NQO1 protects cells from the long-term toxic effects of oxidative injury rather than from the short-term effects of protein and DNA arylation. This idea correlates well with recent findings showing that NQO1 confers protection against oxidative stress by maintaining antioxidant forms of ubiquinone (26) and Vitamin E (27). Much more work is needed to determine exactly how NQO1 confers protection against benzene and other xenobiotics. Fortunately, new molecular tools are available to assist us in this endeavor, including the cell lines described above and a transgenic knockout mouse that lacks NQO1 (28). This NQO1 knockout mouse is more susceptible to the toxic effects of menadione and should provide an excellent model for benzene research and mechanistic studies of the role of NQO1 in cellular protection.

An early observation, of great importance for future research, was made by Huggins and Fukunishi in the early 1960s (29). They showed that low doses of polycyclic aromatic hydrocarbons or azo dyes protected rats from carcinogenesis by high doses of these same chemicals and caused a simultaneous increase in liver menadione reductase, later identified as NQO1. Many different classes of compounds have now been shown to induce NQO1 and can be categorized into monofunctional and bifunctional inducers (3). Bifunctional inducers, such as dioxin and aromatic hydrocarbons, induce NQO1 via the Ah receptor and the xenobiotic response element. Monofunctional inducers appear to act through the antioxidant response element and the redox-sensitive proteins fos and jun (30, 31) and include hydrogen peroxide (32) and phenolic antioxidants (33). It seems likely that hydroquinone and other benzene metabolites induce NQO1 in bone marrow via the antioxidant response element, because incubation of myeloid

cells with hydroquinone increases hydrogen peroxide production (34) and active oxygen species are increased in the bone marrow after benzene exposure (35). Induction of NQO1 through the antioxidant response element may therefore serve to protect cells against the damaging effects of active oxygen species and other forms of oxidative stress. Again, this idea fits well with NQO1 playing a general role in protecting cells from the secondary effects of chemical exposure.

Because NQO1 induction appears to protect against chemical carcinogenesis (5) and mutagenesis (36, 37), it would seem logical that individuals lacking NQO1 activity because of inheritance of homozygous mutant (T/T) alleles would be at higher risk of developing certain cancers. However, the molecular epidemiological studies that have been performed to date have produced mixed results. An increased risk of urological malignancies has been associated with the T/T genotype (38), but no increased risk of prostate cancer was found (39), and the association between lack of NQO1 activity and lung (40, 41) and colon cancer (42, 43) remains controversial. Clearly more studies are needed, preferably with larger numbers of cases to increase study power.

Given the association between lack of NQO1 activity, benzene toxicity, and subsequent risk of benzene-induced leukemia, my laboratory has decided to investigate the role of the NQO1 609 C → T polymorphism in leukemia in general. Together with Richard Larson and colleagues, we studied a series of 104 leukemia cases from the Chicago area, more than half of which had myeloid leukemia secondary to chemotherapy (t-AML) (44). The mutant allele frequency was 1.4-fold higher than expected in the t-AML cases and was 1.6-fold higher among patients with abnormalities in chromosomes 5 and/or 7. Interestingly, we have recently shown that benzene increases abnormalities in chromosomes 5 and 7 in exposed workers (45), and hydroquinone produces similar changes in cultured human cells (46). Thus, lack of or lowered NQO1 activity may make individuals vulnerable to leukemia secondary to chemical exposure. My laboratory is currently investigating this issue further in case-control studies of leukemia in adults in the United Kingdom, in collaboration with Gareth Morgan and Eve Roman, and in children in California, with Patricia Buffler and John Wiencke.

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