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Ethnic variation in the prevalence of a common NAD(P)H quinone oxidoreductase polymorphism and its implications for anti-cancer chemotherapy

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Summary The NAD(P)H quinone oxidoreductase (NQO1:EC 1.6.99.2) is an important biotransformation enzyme system that is also known to metabolize important novel chemotherapeutic compounds. The gene that codes for this enzyme has recently been found to be polymorphic in humans. Here, we describe the ethnic distribution of the polymorphism and note that this may have implications for anti-tumour drug development and use.

Keywords: polymorphism; NQO1; DT Diaphorase; ethnicity; second cancer

The ability of NAD(P)H quinone oxidoreductase (NQO1: EC 1.6.99.2) to bioactivate anti-tumour quinones as well as the elevated NQO1 activity in certain tumours has led to a considerable focus on NQO1 in enzyme-directed drug development (Riley and Workman, 1992; Workman, 1994; Ross et al, 1993, 1994; 1996a). NQO1 has been shown to bioactivate both mitomycin C (Siegel et al, 1990) and EO9 (Walton et al, 1991). Correlations between NQO1 activity and either mitomycin C or EO9 sensitivity have been described in cellular systems (Riley and Workman, 1992; Workman, 1994; Ross et al, 1996) but, more recently, significant correlations between NQO1 activity and mitomycin C and EO9 sensitivity have been demonstrated in 69 cell lines in the NCI human tumour cell line panel (Fitzsimmons et al, 1996). The cytotoxicity of streptonigrin has also been reported to exhibit an excellent correlation with NQO1 activity in cell lines of the NCI panel (Paul et al, 1994). One significant implication of this work is that knowledge of a particular tumour's level of activating or deactivating enzymes may help in the selection of patients to receive specific anti-cancer therapies, and thereby achieve improved therapeutic selectivity. An important consideration in the development of these approaches is the possible effects of inherited variation in the genes encoding bioreductive enzymes. A further aspect in assessing the general applicability of these strategies is the genetic differences related to the racial and ethnic constitution of patient populations.

Recently, a C to T transition at basepair 609 of exon 6 in the gene encoding NQO1 has been described (Traver et al, 1992; Rosvold et al, 1995; Ross et al, 1996). The variant allele is thought to code for a proline to serine amino acid substitution in codon 187 and is associated with a loss of NQO1 protein and enzyme activity (Traver et al, 1992; Rosvold et al, 1995; Ross et al, 1996). The polymorphic NQO1 enzyme is a dimeric FAD-containing cytosolic protein that catalyses the two-electron reduction of a variety of quinone compounds (Riley and Workman, 1992; Workman, 1994; Ross et al, 1993, 1994). NQO1 functions as a mechanism for the reductive activation of a growing number of chemotherapeutic agents (Table 1).

METHODS

Because of the increasingly prominent role of bioreductive drugs in cancer chemotherapy and the potential for genetic heterogeneity, we examined the ethnic variation in the prevalence of the NQO1 polymorphism in 529 healthy subjects including Caucasians, Hispanics, African-Americans and Asians.

We used a polymerase chain reaction (PCR)-based approach for genotyping; briefly, a 211-bp PCR fragment was amplified from DNA isolated from whole blood using the sense primer (5'-TCTCAGAGTGGCATTCTGC-3') and antisense primer (5'-TCTCCTCATCCTGTACCTCT-3'). The variant allele is detected using a *HinfI* restriction digest run on 1.8% agarose gels. The African-American, Mexican-American and Caucasian individuals were healthy adult control subjects participating in lung cancer case-control studies. The Asian participants were healthy workers in Korea or participants in a longitudinal study of reproduction in mainland China.

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Table 1 Chemotherapeutic agents metabolized by NQO1

Mitomycin C	Diaziquone
Tirapazamine ^a	Streptonigrin
EO9	PDZQ
Porofiromycin	MeDZQ
Mitoxantrone	CB1954
Ametantrone	

PDZQ, 2,5-diaziridinyl-3-phenyl-1,4-benzoquinone; MeDZQ, 2,5-diaziridinyl-3,6-dimethyl-1,4-benzoquinone; EO9, 3-hydroxymethyl-5-aziridinyl-1-methyl-2-(1H-indole-4,7 dione)-propenol; CB1954, 5-(aziridin-1-yl)-2,4-dinitrobenzamide. ^aAll compounds are bioactivated by NQO1 except tirapazamine, which is detoxified by NQO1.

Table 2 NQO1 genotype frequencies in different ethnic groups

Ethnic group	Wt*/Wt (%)	Wt/V** (%)	V/V (%)	Allele frequency (Wt) (V)
Non-Hispanic White (n = 114)	64 (56.1)	45 (39.5)	5 (4.4) ^a	(0.75) (0.25)
Mexican Hispanic (n = 161)	52 (32.3)	84 (52.2)	25 (15.5) ^b	(0.57) (0.43)
African-American (n = 136)	83 (61.0)	46 (33.8)	7 (5.2) ^c	(0.78) (0.22)
Asian (n = 118)	37 (31.4)	57 (48.3)	24 (20.3) ^d	(0.56) (0.44)
Korean (n = 69)	23 (33.3)	33 (47.8)	13 (18.8)	(0.58) (0.42)
Chinese (n = 49)	14 (28.6)	24 (50.0)	11 (22.4)	(0.53) (0.47)

Numbers in parentheses do not always add up to 100 because of rounding. *Wt, wild-type allele; **V, variant allele. ^aSignificantly different from ^b($P < 0.05$) and ^c($P < 0.05$). ^dSignificantly different from ^b($P < 0.05$) and ^c($P < 0.05$).

RESULTS

The prevalence of the NQO1 polymorphism in different ethnic groups is shown in Table 2. We found that approximately 5% of African-Americans were homozygous for the variant NQO1 allele. Interestingly, 5% of the Caucasians were similarly homozygous for this trait. The Asian population had the highest frequency of homozygous variant individuals with 20.3% overall (18.8% in Koreans and 22.4% in Chinese). The Mexican-American volunteers were only slightly lower in homozygous variant prevalence compared with Asians, with 15.5% having two copies of this allele. The prevalence of heterozygous individuals ranged from 34% to 52%. All of the allele frequencies were in Hardy-Weinberg equilibrium.

DISCUSSION

These results demonstrate that the variant allele of the NQO1 gene is remarkably common in all ethnic groups examined to date. It has been shown that homozygous variant cells have a complete absence of NQO1 protein and activity (Traver et al, 1992; Rosvold et al, 1995; Ross et al, 1996). Therefore, our results would predict that 5–20% of patients (depending upon ethnicity) will have diminished metabolic activation of bioreductive compounds. This could adversely affect the outcome of such therapies because the tumour-specific action of these agents is thought to depend on

increased expression of NQO1 in tumours compared with normal tissue. Unlike carriers of two of the variant alleles, heterozygotes may have intermediate sensitivity of normal tissue while retaining enhanced enzyme expression in tumours. The precise effect of different NQO1 genotypes is unknown. Consequently, it is crucial that well-controlled studies be carried out to define the phenotypic effects of heterozygote and homozygote NQO1 expression, as this understanding may afford novel opportunities to increase the efficacy of bioreductive chemotherapeutics.

Because NQO1 can potentially affect the generation of toxic as well as therapeutic intermediates, genetic variations that affect NQO1 activity may impact upon both the acute and chronic side-effects of cancer treatments. Increased myelosuppression associated with exposure to the known leukaemogen, benzene, occurs in persons carrying the variant NQO1 gene (Rothman et al, 1996). Hence, the dose-limiting toxicity of bioreductive drugs could also be affected by the NQO1 polymorphism, and could vary with its ethnic distribution. Long-term complications of cancer chemotherapy include the induction of second tumours (Platz et al, 1996). For example, combination therapy for breast cancer including mitomycin C and mitoxantrone is known to carry a risk for secondary leukaemia (Philpott et al, 1993). Investigation of the association of NQO1 genotypes with adverse outcome could assist in ongoing efforts to select those patients most likely to benefit from a particular drug or combination of drugs.

Although our data show that the NQO1 polymorphism is common, they also indicate significant ethnic differences in allele frequencies; homozygous variants were fourfold more common among Asians compared with Caucasians. Importantly, it is also well known that, for some cancers, the success of treatment is influenced by race and ethnicity (Elledge et al, 1994; Modiano et al, 1995). For example, Berenberg (1991) has suggested that differences in drug distribution, elimination and metabolism related to genetics could explain the variability in outcome of treatment for breast carcinoma identified between Asian and Caucasian women. Our results emphasize the importance of considering the genetic heterogeneity of diverse patient populations in developing and evaluating bioreductive agents used in cancer chemotherapy.

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