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POLYMORPHISMS OF MICROSOMAL EPOXIDE HYDROLASE IN FRENCH VINYL CHLORIDE WORKERS

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Abstract
Objectives: The purpose of this study was to determine if polymorphisms in microsomal epoxide hydrolase, an enzyme involved in the metabolism of reactive intermediates of vinyl chloride (VC), contribute to the variable susceptibility to the mutagenic effects of vinyl chloride among exposed workers. **Materials and Methods:** Polymorphisms at codons 113 and 139 were determined in DNA samples from 211 French vinyl chloride workers. Genotypes were stratified into low, medium and high activity groups and odds ratios and 95% confidence intervals were determined for the presence of one or both of two VC-induced mutant biomarkers (mutant ras-p21 and mutant p53) by logistic regression adjusting for age, smoking, drinking and cumulative VC exposure. **Results:** Compared to the low-activity microsomal epoxide hydrolase genotype stratum, the odds ratio for the presence of the VC-induced mutant biomarkers increased to 1.16 (95% CI: 0.64–2.10) in the medium-activity genotype stratum and to 1.35 (95% CI: 0.66–2.77) in the high-activity genotype stratum. The test for trend was not statistically significant and was in the opposite direction from that expected based on increasing removal of reactive intermediates with increasing activity. **Conclusions:** The results suggest that polymorphisms in microsomal epoxide hydrolase do not play a significant role in susceptibility to the mutagenic effects of vinyl chloride.

Key words:
Vinyl chloride, Mutations, Response biomarkers, Susceptibility biomarkers

INTRODUCTION

Vinyl chloride (VC) is a known animal and human carcinogen associated with a rare sentinel neoplasm, angiosarcoma of the liver [1]. The metabolism of VC involves the production of reactive intermediates capable of damaging DNA, including the production of specific mutations in cancer-related genes such as *ras* and p53, which are believed to be critical steps in the VC carcinogenic pathway [2]. For example, the initial step in the metabolism of VC in the liver involves the action of CYP2E1 to produce the reactive intermediates chloroethylene oxide (CEO) and chloroacetaldehyde (CAA), which are capable of binding to DNA generating oxoethyl- and etheno-adducts, with the etheno-adducts being capable of producing

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the specific mutations noted in cancer-related genes [1,2]. Metabolism of these reactive intermediates is thought to involve several pathways that rely on aldehyde dehydrogenase 2 (ALDH2), glutathione-S-transferases (GSTs), microsomal epoxide hydrolase (mEH) and other enzymes, presumably to generate less reactive metabolites for excretion [1]. All of these enzymes (CYP2E1, ALDH2, GSTs and mEH) are known to have polymorphic variants with altered activities that could produce variable VC metabolism [3]. Such variable metabolism could account for differing susceptibilities to the carcinogenic effects of VC in exposed individuals.

In a study of French VC workers, we have previously identified a dose-dependent increase in circulating biomarkers for *ras* and p53 mutations presumably indicative of VC-induced genetic alterations [4,5]. However, among these workers, individuals with estimated similar exposures could have different patterns of expression of these mutant biomarkers. We have therefore begun to examine these workers for polymorphisms in VC metabolism or repair genes to explain this variability in mutagenic response to similar VC exposures. For example, in this cohort we found no individuals with the ALDH2 polymorphism, and polymorphisms in GSTM1, GSTT1 and GSTP1 did not contribute significantly to biomarker variability [6,7]. We did find that individuals with the high activity CYP2E1 c2 allele had a statistically significantly increased risk for the presence of the mutant biomarkers, even after adjusting for confounders such as age, smoking, alcohol drinking and cumulative VC exposure, compared to homozygous wild-type individuals [6]. However, this polymorphism occurred in only 7.6% of the cohort so it could only account for a small proportion of the biomarker variability [6]. Therefore, in this study, we have examined these workers for the more common polymorphisms in mEH to determine if they contribute to variability in response to VC exposure.

MATERIALS AND METHODS

Subjects for study were selected from a previously described population of VC-exposed workers in France [4]. A cohort of 225 of these workers had been previously analyzed for circulating mutant *ras*-p21 and mutant p53 biomarkers in their serum [4,5]. A group of 211 of these workers with available lymphocytes for DNA extraction were selected for the study. The study protocol was reviewed and approved by the Columbia University Institutional Review Board for conformance with human subjects protections. All of the workers in the study were white males with the following characteristics: average age = 56 years (range = 35–74); average cumulative exposure = 5871 ppm-years (range = 6–46,702); 39.3% current or former smokers; 19.9% current alcohol drinkers; 62% positive for at least one mutant biomarker (44.5% positive for mutant p53, 36.5% positive for mutant *ras*-p21, 19% positive for both).

From blood samples from each of the workers, lymphocytes were cultured and DNA extracted by routine techniques. Each DNA sample was analyzed for the presence of two different mEH polymorphisms by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques, as previously described [8], to detect the T to C transition in exon 3 (Tyr113His) and the A to G transition in exon 4 (His139Arg). Briefly, for PCR, 20 ng of DNA was amplified in a total volume of 10 μL of buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.4 mM of the forward and reverse primers (5'-GATCGATAAGTTCCGTTTCACC-3' and 5'-AATCTTAGTCTTGAAGTGAGGAT-3' for exon 3 and 5'-ACATCCACTTCATCCACGT-3' and 5'-ATGCCTCTGAGAAGCCAT-3' for exon 4 and 0.3 U Taq polymerase. The mixture underwent initial denaturation at 94°C for 5 min followed by 38 cycles (for exon 3) or 34 cycles (for exon 4) at 94°C for 20 sec, annealing at 55°C for 30 sec and elongation at 72°C for 50 sec. The resulting PCR products were subjected to restriction cleavage (EcoRV for exon 3 and RsaI for exon 4) with the fragments separated by 2.6% agarose gel electrophoresis (for exon 3) and 2.2% agarose gel electrophoresis (for exon 4), stained with ethidium bromide and visualized under UV light. By this approach, individuals were classified for the exon 3 codon 113 polymorphisms as homozygous wild-type (Tyr/Tyr; bands of 140 bp and 20 bp), heterozygous

(Tyr/His; bands of 162 bp, 140 bp and 20 bp) or homozygous variant (His/His; band of 162 bp) and for the exon 4 codon 139 polymorphism as homozygous wild-type (His/His; band of 210 bp), heterozygous (His/Arg; bands of 210 bp, 164 bp and 46 bp) or homozygous variant (Arg/Arg; bands of 164 bp and 46 bp).

In vitro analyses of the resultant proteins with these polymorphisms have demonstrated a 40% decrease in activity in the Tyr113His variant and a 25% increase in activity in the His139Arg variant [9]. Based on these enzyme activity profiles, individuals with combinations of alleles in their exon 3 and exon 4 genotypes have been classified according to their expected mEH activity as follows [10]:

- low activity: His/His-His/His; His/His-His/Arg; Tyr/His-His/His; and His/His-Arg/Arg;
- medium activity: Tyr/Tyr-His/His; Tyr/His-His/Arg; and Tyr/His-Arg/Arg;
- high activity: Tyr/Tyr-Arg/Arg; Tyr/Tyr-His/Arg.

Based on this classification scheme, the workers were stratified by low, medium or high mEH activity and the odds ratio and 95% CI were calculated (adjusted for age, smoking, alcohol drinking and cumulative VC exposure) for the presence of one or both of the mutant biomarkers.

RESULTS

Among the 211 workers, 123 (58%) were homozygous wild-type (Tyr/Tyr), 63 (30%) were heterozygous (Tyr/His), and 25 (12%) were homozygous variant (His/His) at codon 113; 136 (64%) were homozygous wild-type (His/His), 69 (33%) were heterozygous (His/Arg), and 6 (3%) were homozygous variant (Arg/Arg) at codon 139. For codon 139, these results were consistent with a Hardy-Weinberg equilibrium and with those found in comparable populations [11–13]. For codon 113, the results were consistent with those found in comparable populations but were not in a Hardy-Weinberg equilibrium, which has been noted in other studies [11–13]. Consistency of this finding across studies suggest that this is not due to methodological problems but rather may represent unexplained genetic instability. There were no significant differences in terms of the distribution of exposure levels among these workers by genotype.

Table 1. Association between the mEH codon 113 polymorphism and mutant *ras*-p21 and mutant p53 in VC workers

mEH 113	p21 and p53 biomarkers			Odds Ratio* (95% CI)
	Both –	Either +	Both +	
Tyr/Tyr (n = 123)	45 (37%)	51 (41%)	27 (22%)	1.0
Tyr/His (n = 63)	23 (37%)	31 (49%)	9 (14%)	0.87 (0.48–1.56)
His/His (n = 25)	12 (48%)	9 (36%)	4 (16%)	0.60 (0.26–1.36)

* Adjusted for age, smoking, alcohol drinking and cumulative VC exposure; test for trend, p = 0.23.

Table 2. Association between the mEH codon 139 polymorphism and mutant *ras*-p21 and mutant p53 in VC workers

mEH 139	p21 and p53 biomarkers			Odds Ratio* (95% CI)
	Both –	Either +	Both +	
His/His (n = 136)	51 (38%)	59 (43%)	26 (19%)	1.0
His/Arg (n = 69)	27 (39%)	29 (42%)	13 (19%)	0.98 (0.57–1.70)
Arg/Arg (n = 6)	2 (48%)	3 (50%)	1 (17%)	1.15 (0.25–5.32)

* Adjusted for age, smoking, alcohol drinking and cumulative VC exposure; test for trend, p = 0.97.

Table 3. Association between mEH activity based on polymorphisms at codons 113 and 139 and mutant *ras*-p21 and mutant p53 in VC workers

mEH activity	p21 and p53 biomarkers			Odds Ratio* (95% CI)
	Both –	Either +	Both +	
Low (n = 54)	26 (42%)	27 (42%)	11 (17%)	1.0
Medium (n = 102)	38 (372%)	45 (44%)	19 (19%)	1.16 (0.64–2.10)
High (n = 45)	16 (36%)	19 (42%)	10 (22%)	1.35 (0.66–2.77)

* Adjusted for age, smoking, alcohol drinking and cumulative VC exposure; test for trend, p = 0.41.

In terms of combined genotypes, 19 (9%) were His/His-His/His, 6 (3%) were His/His-His/Arg, 39 (18%) were Tyr/His-His/His, and 0 (0%) were His/His-Arg/Arg, for a total of 54 individuals with a presumed low activity phenotype; 78 (37%) were Tyr/Tyr-His/His, 22 (10%) were

Tyr/His-His/Arg, and 2 (1%) were Tyr/His-Arg/Arg, for a total of 102 individuals with a presumed medium activity phenotype; and 4 (2%) were Tyr/Tyr-Arg/Arg and 41 (19%) were Tyr/Tyr-His/Arg, for a total of 45 individuals with a presumed high activity phenotype.

As presented in Tables 1 and 2, the results of the association between the individual polymorphisms at codon 113 and 139 and the mutant biomarkers show no consistent or significant pattern. The results of the association between combined mEH activity strata and mutant biomarkers are presented in Table 3. In the low activity stratum, 26 (41%) workers were negative for both biomarkers, 27 (42%) workers were positive for one of the biomarkers, and 11 (17%) workers were positive for both biomarkers. In the medium activity stratum, 38 (37%) workers were negative for both biomarkers, 45 (44%) workers were positive for one biomarker, and 19 (19%) workers were positive for both biomarkers. In the high activity stratum, 16 (36%) workers were negative for both biomarkers, 19 (42%) workers were positive for one biomarker, and 10 (22%) workers were positive for both biomarkers. Assigning an odds ratio of 1 to the low activity stratum yields an adjusted odds ratio of 1.16 (95% CI: 0.64–2.10) for biomarker positivity in the medium activity stratum and an adjusted odds ratio of 1.35 (95% CI: 0.66–2.77) for biomarker positivity in the high activity stratum, although this trend was not statistically significant ($p = 0.41$). These results were essentially unchanged when other proposed classification schemes for genotype based on presumed mEH activity [14] were employed.

DISCUSSION AND CONCLUSIONS

These results suggest that there is no statistically significant effect of mEH genotype on biomarkers of VC-induced mutagenic damage, and thus mEH may not be involved as a major detoxification enzyme in VC metabolism in humans. We had hypothesized that mEH would be important for the detoxification of VC reactive intermediates based on prior *in vitro* and animal studies that supported this assumption [15,16]. Conversely, other studies have demonstrated that, although mEH may be involved to some degree in CEO metabolism, blockage of mEH activity with a specific inhibitor did not enhance the level of VC-induced adenine adducts [17]. Since etheno-adenine adducts are responsible for the VC-induced mutations in p53, this latter finding would be consistent with our observation of no significant effect of varying mEH activity, as determined by the exon 3 and 4 polymorphisms, on the occurrence of biomarkers of VC-induced mutagenic damage, including the biomarker for p53 mutations.

In addition, the prior studies on the role of mEH in detoxification of VC reactive intermediates [15,16] led us to hypothesize that increased mEH activity would lead to increased detoxification and decreased reactive intermediates, so we expected to observe a decreasing trend in the occurrence of the biomarkers from the low to the medium to the high activity strata. Surprisingly, if there is any trend, it appears to be in the opposite direction, i.e. an increase in biomarker positivity with increasing mEH activity. This does not seem to be due to the particular genotype-activity classification scheme employed, since, as noted, the use of other schemes did not affect the results. The effect of mEH genotype on metabolism has not been investigated in other VC-exposed cohorts. However, there are other studies of the effect of mEH genotype on the metabolism of other carcinogens and on cancer risk. For example, one study found a significant increase in DNA damage in human lymphocytes exposed to styrene in individuals with a medium activity mEH phenotype compared to a high activity mEH phenotype; however, the DNA damage in the medium activity phenotype group was also significantly higher than in the low activity phenotype group, indicating no consistent trend [18]. mEH has also been implicated in the metabolism of the reactive intermediates of polycyclic aromatic hydrocarbon (PAH) carcinogens, such as benzo(a)pyrene diolepoxide (BPDE); but it has been noted that in this case mEH can have dual functions, acting to inactivate BPDE or contributing to the activation of BPDE to its highly reactive ultimate metabolite, making it difficult to interpret the effect of mEH activity on levels of BPDE-DNA adducts in the leukocytes of smokers [14]. However, high activity mEH genotypes have been associated with increased risk of developing PAH-related malig-

nancies in some studies [19,20]. On the other hand, a study found that individuals with a high level of smoking and charred meat intake (i.e. high PAH exposure) with low activity mEH genotypes were at elevated risk of colorectal adenomas [21]. Other studies have found no significant effect of mEH polymorphisms on colorectal adenoma risk [13]. Therefore, there seems to be little consistency among studies concerning the potential effects of mEH polymorphisms and activity on carcinogen metabolism and cancer risk, and the outcomes may vary, depending on the particular carcinogen or type of cancer being examined. Finally, it has been suggested that the exon 3 and 4 polymorphisms in mEH may account for only a modest amount of overall enzyme activity in comparison to inter-individual differences in mEH expression ranging from 2- to 10-fold [13], and there may exist polymorphic loci in the regulatory part of the mEH gene that could account for this variability in expression [22]; until such polymorphisms can be taken into account, it may be difficult to obtain coherent results based on the exon 3 and 4 polymorphisms alone.

On the basis of the results in this study, it appears that mEH polymorphisms at exon 3 and 4 alone do not play a significant role in the variation in mutagenic damage occurring in response to VC exposure. Other factors, such as genetic variation in DNA repair capacity [23], may be much more significant in explaining the observed susceptibility patterns.

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