METABOLIC ACTIVATION/INACTIVATION

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The GST T1 and CYP2E1 genotypes are possible factors causing vinyl chloride induced abnormal liver function

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Abstract Vinyl chloride monomer (VCM) is hepatotoxic as well as carcinogenic in humans. There are reports that exposure to VCM seems to induce abnormal liver function, liver fibrosis, cirrhosis, portal hypertension, and angiosarcoma of the liver. In vivo, VCM is metabolized by cytochrome P450 2E1 (CYP2E1) to form the electrophilic metabolites, chloroethylene oxide (CEO) and chloroacetaldehyde (CAA), which may either cause cell damage or be further metabolized and detoxified by glutathione S-transferases (GSTs). This study investigated whether or not the genotypes CYP2E1, glutathione S-transferase θ (GST T1) and μ (GST M1) correlated with abnormal liver function found in vinyl chloride exposed workers. For this study, 251 workers from five polyvinyl chloride plants were enrolled. The workers were classified into two exposure groups (high and low) and the degree of exposure was determined based on their job titles and airborne VCM concentration. The activity of serum alanine aminotransferase (ALT) was used as the parameter of liver function. The genotypes CYP2E1, GST T1 and GST M1 were determined by polymerase chain reaction and restriction fragment length polymorphism on peripheral white blood cell DNA. Other potential risk factors were also ascertained and the confounding effect was adjusted accordingly. Stratified analyses were used to explore the correlation between the alteration of liver function and the genotypes CYP2E1, GST T1 and GST M1 among the workers exposed to different levels of VCM. The following results were obtained (1) at low VCM exposure, the odds ratio (OR) of positive GST T1 on ab-

normal ALT was 3.8 (95% CI 1.2–14.5) but the CYP2E1 genotype was not associated with abnormal ALT. (2) At high VCM exposure, a c2c2 CYP2E1 genotype was associated with increased OR on abnormal ALT (OR 5.4, 95% CI 0.7–35.1) and positive GST T1 was significantly associated with decreased OR on abnormal ALT (OR 0.3, 95% CI 0.1–0.9). (3) Multiple linear and logistic regression also showed strong interactions of the VCM exposure to CYP2E1 as well as to the GST T1 genotype. These observations suggest that the two genotypes, CYP2E1 and GST T1, may play important roles in the biotransformation of VCM, the effect of which leads to liver damage.

Key words Vinyl chloride · Glutathione S-transferases · Cytochrome P-450 2E1 · Liver function

Introduction

Vinyl chloride monomer (VCM) has been associated with hepatotoxic effects in humans including liver fibrosis, cirrhosis, hepatomegaly, splenomegaly, portal hypertension, and angiosarcoma of the liver (Creech and Johnson 1974; Lilis et al. 1975; Smith and Crossley 1976; Simonato et al. 1991; Wang et al. 1991). Exposure to VCM may also cause abnormalities in one or more liver function tests, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (GGT; Makk et al. 1974; Lilis et al. 1975; Marsteller and Lebach 1975; Ho et al. 1991; Du et al. 1995).

The liver is the primary target of VCM because it is believed that VCM is activated by cytochrome P450 2E1 (CYP2E1), an enzyme mainly existing in the liver (Guenengerich et al. 1991; Koop 1992). CYP2E1 catalyses VCM oxidation into an epoxide intermediate, chloroethylene oxide (CEO), which is spontaneously rearranged into 2-chloroacetaldehyde (CAA). The electrophilic metabolites, CEO and CAA, considered to be the most important intermediates in the VCM carcinogenic

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process react with DNA bases to form adducts. The DNA-adducts are mutagenic in the bacterial system as well as in mammalian cells or covalently bind to proteins to impair cell function (Bolt 1986; Brandt-Rauf et al. 1995). These two metabolites may be further processed by glutathione S-transferases (GSTs; Bolt 1986; Easter and Von Burg 1994). The activities of these enzymes, GSTs and CYP2E1, are known to vary genetically among individuals. Thus, inter-individual differences in susceptibility to VCM exposure may be attributable to the differences in the activities of CYP2E1 and GSTs. Metabolism of VCM may proceed via different pathways at different concentrations (Easter and Von Burg 1994). The pathway described above mainly proceeds at high concentration. At low concentration, VCM appears to be oxidized by alcohol dehydrogenase to form chloroethanol, CAA and chloroacetic acid. The latter two products may further conjugate with glutathione (GSH; Easter and Von Burg 1994). Therefore, the susceptibility of an individual to the induction of liver damage may depend on the exposure concentration of VCM. In other words, individuals with the same genetic trait exposed to different levels of VCM may exhibit different health effects.

The genetic variation in CYP2E1 is supported by recent identification of the restriction fragment length polymorphisms (RFLPs) of the CYP2E1 5'-flanking region detected by *Pst*I and *Rsa*I digestion. The two restriction sites, *Pst*I and *Rsa*I, are tightly linked and such genotype may affect the transcriptional regulation of CYP2E1 (Hayashi et al. 1991; Watanabe et al. 1994). The relationship has been reported between CYP2E1 genetic polymorphisms and susceptibility to lung cancer (Perrson et al. 1993), alcoholic liver disease (Maezawa et al. 1994; Tsutsumi et al. 1994; Chao et al. 1995), hepatocellular carcinoma (Yu et al. 1995) and nasopharyngeal carcinoma (Hildesheim et al. 1995) respectively.

Concerning GSTs, the mammalian GST supergene family, composed of α , π , μ , and newly discovered θ , catalyses the conjugation of GSH with electrophilic substrates. Some individuals carry a large deletion (null genotype) in the glutathione S-transferase θ (GST T1) and glutathione S-transferase μ (GST M1) gene, respectively. An individual without an intact GST M1 may have a higher risk of developing cancer or cytogenetic damage (Wiencke et al. 1990; Seidegard et al. 1986, 1990). The role of the newly discovered GST T1 remains controversial. An *in vitro* study shows that GST T1 deficiency may increase chromosome damage by ethylene oxide (Hallier et al. 1993) or by diepoxybutane (Norppa et al. 1995). On the other hand, there are some classes of agents (e.g. halomethanes) that are activated by conjugating with GSH to form mutagenic intermediates (Thier et al. 1993). Thus, different substrates metabolized by GST T1 may have different consequences (Hallier et al. 1993; Norppa et al. 1995; Thier et al. 1993; Nelson et al. 1995).

Several studies performed in the 1970s showed a higher prevalence of abnormal liver function in VCM

exposed workers (Lilis et al. 1975; Makk et al. 1974; Marsteller and Lebach 1975). However, other studies indicate that the liver function tests used are not sensitive enough to detect early VCM-induced liver damage (Williams et al. 1976; Sugita et al. 1986). Although AST, ALT and GGT have been proposed as indicators of liver damage, their specificity and sensitivity remain unknown. Ho et al. (1991) report that ALT is the earliest and most sensitive indicator of VCM-induced liver dysfunction followed by GGT. It is believed that ALT is more specific than AST as an indicator of liver damage since ALT is found primarily in the liver, whereas AST is also present in many other tissues including heart, skeletal muscle, kidney and brain (Podolsky and Isselbacher 1994). Because ALT is more sensitive and specific than AST or GGT, and more suitable for the screening of occupational toxic exposure (Redlich and Brodtkin 1994; Widmann 1989), ALT was used as an indicator of liver damage in this study.

There may be interactions between the susceptible factors and chemical exposure. Hence, the magnitude and direction of associations between exposures and diseases may differ according to the extent of genetic susceptibilities (Vine and McFarland 1990). On the other hand, the effect of genetic susceptibilities may depend on the exposure concentrations when the metabolism of the chemical proceeds via different pathways at different concentrations. In this study, we attempted to determine whether or not there were interactions between the genotypes of CYP2E1, GST T1, and GST M1 and the VCM exposure in inducing liver damage.

Materials and methods

Subjects

Two hundred and eighty volunteers were recruited from five polyvinyl chloride (PVC) plants. Female workers ($n = 13$) were excluded from this study because of insufficient number. Those who were newly employed or changed workplace within 6 months ($n = 13$) were also excluded, on consideration that the time for the induction of liver damage from VCM exposure might not be sufficient (Marsteller and Lebach 1975). Some data were missing from three workers, who were likewise excluded. Thus, a total of 251 male workers was employed for the final analysis. All workers received complete physical examinations and structured questionnaires including demographic data, smoking and drinking habits, and detailed occupational history. Questionnaires were administered by well-trained interviewers.

Subjects were divided into two exposure groups, high and low, according to the nature of their current job. The high-exposure group was defined as those individuals involved in unloading PVC or VCM, adding catalyst, tank cleaning, working in a control room, tank stripping, and being a foreman; the low-exposure group were those individuals involved in the drying operation, laboratory testing, managing industrial hygiene, machine maintenance, PVC storage, gatehouse keeping, administration and car driving. Exposure assessment performed 1 year before this study showed that the 8 h time-weighted average (TWA) of VCM in the low-exposure group was always ≤ 1 ppm, while the TWA of VCM in the high-exposure group ranged from 1 to 5 ppm; occasionally concentrations up to 40 ppm were found in certain jobs including unloading of PVC, adding of catalyst and tank cleaning.

Samples

Ten millilitres of venous blood were drawn into heparinized tubes (Vacutainer) and stored at 4 °C. The whole blood was separated into plasma, buffy coat, and red blood cells by centrifugation within 18 h of obtaining the blood, then stored in a -70 °C freezer. Genomic DNA was extracted and purified from buffy coat as previously described (Hsieh et al. 1996).

Measurement of ALT

ALT was analysed with a Hitachi 7050 autoanalyser. Enzyme immunoassay (Austria-II, Abbott Lab., Chicago, IL., USA) was used to determine the presence or absence of the serum hepatitis B virus surface antigen (HBs Ag) and anti-hepatitis C virus antibody (anti-HCV Ab). All these tests were performed in the central laboratory of the National Taiwan University Hospital; the cut-off value of 31 IU/l adopted here for the abnormality of the ALT was that used by the central laboratory.

Genotyping of the GST T1 and GST M1 gene

A simple assay based on polymerase chain reaction (PCR) technology was available to determine the presence or absence of GST T1 (Pemble et al. 1994) and GST M1 (Comstock et al. 1990). Briefly, PCR was performed in a 25 µl mixture containing the buffer supplied by Promega (Madison, Wis., USA), 250 ng of genomic DNA, *Taq* DNA polymerase (1 U), four bases (dNTP), and 200 ng of each of the primers for GST T1 (5'-TTCCTT-ACGGTCCTCACATCTC-3' and 5'-CACCGGATCATGGCC-AGCA-3'), GST M1 (5'-TGCCCTACTTGATTGATGGG-3' and 5'-CTGGATTGTAGCAGATCATGC-3') and the internal control, β -globin (5'-CACAAGTGTGTTCACTAGC-3' and 5'-CA-CTTCATCCACGTTACCC-3'). The running conditions were: 30 cycles at 95 °C for 30 s, 61 °C for 15 s, and 72 °C for 1 min. The PCR product was electrophoresed in 6% polyacrylamide gel, stained with ethidium bromide and photographed under UV light. Individuals without an intact GST T1 or GST M1 gene showed no amplification of the 480 bp GST T1 fragment or the 273 bp GST M1 fragment and a positive internal control, the 100 bp β -globin fragment (Fig. 1).

Fig. 1 Representative results of polymerase chain reaction (PCR) of the GST M1 and GST T1 gene from PVC workers. Genomic DNAs were amplified using PCR, and the products electrophoresed in polyacrylamide gels, and stained with ethidium bromide. The 480 and 273 bp DNA fragments correspond to GST T1 and GST M1, respectively, while the 100 bp DNA fragment corresponds to the β -globin gene as internal control (PVC Polyvinyl chloride, GST glutathione S-transferase)

Fig. 2 Representative results of PCR-RFLP analysis of the CYP2E1 gene from PVC workers. Genomic DNAs were amplified using PCR, and the products digested with *Pst*I, electrophoresed in polyacrylamide gels, and stained with ethidium bromide. The 410 bp DNA fragment corresponds to the c1 allele which does not have a *Pst*I site, while the paired fragments of 290 and 120 bp, correspond to the c2 allele containing the *Pst*I site (RFLP Restriction fragment length polymorphism)

Determination of *Pst*I fragment polymorphism of the CYP2E1 gene

To determine the CYP2E1 fragment polymorphism, genomic DNA was amplified in a 20 µl mixture containing 500 ng of genomic DNA, and 160 ng of each primer (5'-CAGTCGAGTCTACAT-TGTC-3' and 5'-TTCATTCTGTCTTCTAACTG-3') flanking the polymorphic *Pst*I site, for 30 cycles (Hayashi et al. 1991) using the running conditions: 95 °C for 30 s, 57 °C for 15 s, and 72 °C for 1 min. The PCR product was digested overnight with *Pst*I (7.5 units) at 37 °C, electrophoresed in 6% polyacrylamide gels, stained with ethidium bromide and photographed under UV light. The PCR product of CYP2E1 digested with restriction enzyme *Pst*I showed three genotypes of CYP2E1: type c1c1 was not digested by *Pst*I and showed only one band of 410 bp; heterozygous type c1c2 showed three bands of 410, 290 and 120 bp; and type c2c2 showed 2 bands of 290 and 120 bp by polyacrylamide gel electrophoresis (Fig. 2).

Statistical analysis

Statistical Analysis System (SAS) version 6.10 and EGRET (Epidemiologic Graphic, Estimation, and Testing package) were used for the statistical analysis. χ^2 -test was used to compare the difference of basic characteristics between the high- and low-VCM exposure groups. To assess the effect of potential risk factors on liver function, HBs Ag, anti-HCV Ab, habitual alcohol drinking (habitual drinking of at least once a week), age, employment duration, body mass index (BMI), VCM exposure and the genotypes GST T1, GST M1, and CYP2E1 were included in the univariate analyses. Stratified analyses were used to explore the correlation between the alteration of liver function and the genotypes, CYP2E1, GST T1 and GST M1, among the workers exposed to different levels of VCM. Subsequently, a multiple logistic regression model was used to estimate the adjusted odds ratio (OR) of the above risk factors and the effect on abnormal liver function of interactions between the genotypes GST T1, GST M1, and CYP2E1 and alcohol drinking (i.e. habitual alcohol drinking $\times$ GST T1, GST M1 or CYP2E1) or VCM exposure, (i.e. VCM exposure $\times$ GST T1, GST M1 or CYP2E1). The stepwise and backward procedures were used to select the best model. Similar procedures were also used to select the best multiple linear regression model.

Results

The basic characteristics of 251 male workers are shown in Table 1. The high-VCM exposure group seemed to have a higher prevalence of habitual alcohol drinking than the low-VCM exposure group ($\chi^2 = 7.426$, $P < 0.01$); otherwise all the other characteristics of age, BMI, employment duration, and prevalence of positive HBs Ag and anti-HCV Ab, were not statistically different between these two exposure groups. Because the number of positive anti-HCV Ab was small, the data on HBs Ag and anti-HCV Ab were combined and used as the indicator of hepatitis virus infection. When either the HBs Ag or anti-HCV Ab test was positive, the person was scored as positive for virus infection. The genotypic distribution of GST T1, GST M1 and CYP2E1 among the two VCM exposure groups was similar. The total frequency of the null-genotype of GST T1 and GST M1 was 47.4 and 60.6%, respectively. The frequency of the

Table 1 Basic characteristics of the polyvinyl chloride (PVC) workers stratified by vinyl chloride monomer (VCM) exposure (HBs Ag Serum hepatitis B virus surface antigen, anti-HCV Ab anti-hepatitis C virus antibody, GST glutathione S-transferase)

	VCM exposure		
	Low (<i>n</i> = 111) <i>n</i> (%)	High (<i>n</i> = 140) <i>n</i> (%)	Total (<i>n</i> = 251) <i>n</i> (%)
Age			
<40 years	43 (38.7)	70 (50)	113 (45.0)
≥40 years	68 (61.3)	70 (50)	138 (55.0)
Employment duration			
<15 years	46 (41.4)	72 (51.4)	118 (47.0)
≥15 years	65 (58.6)	68 (48.6)	133 (53.0)
Body mass index			
<25 kg/m ²	90 (81.1)	103 (73.6)	193 (76.9)
≥25 kg/m ²	21 (18.9)	37 (26.4)	58 (23.1)
Drinking ^a			
No	104 (93.7)	115 (82.1)	219 (87.3)
Yes	7 (6.3)*	25 (17.9)*	32 (12.7)
HBs Ag			
Negative	91 (82.0)	110 (78.6)	201 (80.1)
Positive	20 (18.0)	30 (21.4)	50 (19.9)
Anti-HCV Ab			
Negative	109 (98.2)	136 (97.1)	245 (97.6)
Positive	2 (1.8)	4 (2.9)	6 (2.4)
Virus infection ^b			
Negative	89 (80.2)	108 (77.1)	197 (78.5)
Positive	22 (19.8)	32 (22.9)	54 (21.5)
GST T1 genotype			
Null	58 (52.3)	61 (43.6)	119 (47.4)
Positive	53 (47.7)	79 (56.4)	132 (52.6)
GST M1 genotype			
Null	70 (63.1)	82 (58.6)	152 (60.6)
Positive	41 (36.9)	58 (41.4)	99 (39.4)
CYP2E1 genotype			
c1c1	61 (55.0)	78 (55.7)	139 (55.4)
c1c2	44 (39.6)	55 (39.3)	99 (39.4)
c2c2	6 (5.4)	7 (5.0)	13 (5.2)

* $\chi^2 = 7.426$, $P < 0.01$

^a Drinking, Habitual alcohol drinking ≥ once a week

^b Virus infection positive, HBs Ag positive or anti-HCV Ab positive

Table 2 Odds ratio (OR; with 95% confidence interval) for abnormal ALT in PVC workers (ALT Alanine aminotransferase)

	Normal <i>n</i> (%)	Abnormal <i>n</i> (%)	OR(95% CI)
VCM exposure			
Low	92 (43.2)	19 (50.0)	1
High	121 (56.8)	19 (50.0)	0.8 (0.4, 1.62)
GST T1 genotype			
Null	101 (47.4)	18 (47.4)	1
Positive	112 (52.6)	20 (52.6)	1.0 (0.5, 2.1)
GST M1 genotype			
Null	129 (60.6)	23 (60.5)	1
Positive	84 (39.4)	15 (39.5)	1.0 (0.5, 2.1)
CYP2E1 genotype			
c1c1/c1c2	204 (95.8)	34 (89.5)	1
c2c2	9 (4.2)	4 (10.5)	2.7 (0.6, 10.2)*
Virus infection			
Negative	176 (82.6)	21 (55.3)	1
Positive	37 (17.4)	17 (44.7)	3.8 (1.7, 8.5)**
Drinking ^a			
Negative	187 (87.8)	32 (84.2)	1
Positive	26 (12.2)	6 (15.8)	1.4 (0.4, 3.7)
Body mass index			
<25 kg/m ²	172 (80.8)	21 (55.3)	1
≥25 kg/m ²	41 (19.2)	17 (44.7)	3.4 (1.5, 7.4)**
Employment duration			
<15 years	100 (46.9)	18 (47.4)	1
≥15 years	113 (53.1)	20 (52.6)	1.0 (0.5, 2.1)

* $P = 0.12$; ** $P < 0.01$; other P -value > 0.1

^a Drinking, Habitual alcohol drinking ≥ once a week

c1c1, c1c2 and c2c2 genotypes of CYP2E1 was 55.4, 39.4 and 5.2%, respectively.

The results of univariate analysis are shown in Table 2. Virus infection [OR (odds ratio) 3.8, $P < 0.01$, 95% CI 1.7–8.5], and BMI (OR 3.4, $P < 0.01$, 95% CI 1.5–7.4) were significantly associated with abnormal ALT. Individuals with CYP2E1 c2c2 genotype had a higher frequency of abnormal ALT but without statistical significance (OR 2.7, $P = 0.12$, 95% CI 0.6–10.2). The factors VCM exposure, the genotypes of GST T1 and GST M1, habitual alcohol drinking, and employment duration, were not associated with abnormal ALT in univariate analyses.

Because the metabolism of VCM may differ at different concentrations, the prevalence of abnormal ALT associated with the genotypes of these metabolic enzymes may be affected by the levels of VCM exposure. In the low-VCM exposure group, the frequency of the GST T1 positive genotype was significantly higher in workers with abnormal ALT (OR 3.8, 95% CI 1.2–14.5) but the CYP2E1 genotype was not associated with abnormal ALT. In the high-VCM exposure group, the frequency of the GST T1 positive genotype was significantly lower in workers with abnormal ALT (OR 0.3, 95% CI 0.1–0.9) and the c2c2 genotype of CYP2E1 was associated with increased OR on abnormal ALT (OR 5.4, 95% CI 0.7–35.1). No association was found between the GST M1 genotype and abnormal ALT whether the VCM exposure was low or high. A significant interaction between the GST T1 genotype and

Table 3 OR (with 95% CI) of abnormal ALT in relation to the GST T1, GST M1 and CYP2E1 genotypes

	VCM exposure					
	Low			High		
	Normal <i>n</i>	Abnormal <i>n</i>	OR (95% CI)	Normal <i>n</i>	Abnormal <i>n</i>	OR (95% CI)
GST T1 genotype						
Null	53	5	1	48	13	1
Positive	39	14	3.8 (1.2, 14.5)**	73	6	0.3 (0.1, 0.9)**
Maentel-Haenszel test for homogeneity, $P < 0.001$						
GST M1 genotype						
Null	58	12	1	71	11	1
Positive	34	7	1.0 (0.3, 3.1)	50	8	1.0 (0.3, 3.1)
Maentel-Haenszel test for homogeneity, $P = 0.96$						
CYP2E1 genotype						
c1c1/c1c2	87	18	1	117	16	1
c2c2	5	1	1.0 (0.0, 9.4)	4	3	5.4 (0.7, 35.1)*
Maentel-Haenszel test for homogeneity, $P = 0.19$						

* $P = 0.053$; ** $0.01 < P < 0.05$; other P -value > 0.1

VCM exposure was indicated based on the analysis using a multiplicative model ($P < 0.001$, Maentel-Haenszel test for homogeneity). Although the OR for the c2c2 genotype was different in the low or high VCM exposure groups, a statistical test for the interaction between the CYP2E1 genotype and VCM exposure indicated non-significance ($P = 0.19$, Maentel-Haenszel test for homogeneity; Table 3)

The data of Table 4 give the best fitted multiple logistic and linear regression models. In the multiple logistic regression model, virus infection (OR 6.4, $P < 0.01$, 95% CI 2.7–15.3), and BMI of ≥ 25 kg/m²

(OR 5.7, $P < 0.01$, 95% CI 2.4–13.7) were significantly associated with increased OR on abnormal ALT. Interactions between VCM exposure and the genotypes of GST T1 and CYP2E1 could be seen. The effects of the GST T1 or CYP2E1 genotypes on inducing liver damage differed with different levels of VCM exposure; therefore, the odds ratio should be discussed separately on each category. Compared to individuals with low VCM exposure and null GST T1, the OR for individuals with combined GST T1 positive and low VCM exposure on abnormal ALT was 7.1 times the null genotype at low VCM (OR 7.1, 95% CI 2.1–24.1, $P < 0.01$), while the

Table 4 Multiple linear and logistic regression model for ALT in PVC workers, $n = 251$ (BMI Body mass index)

	Logistic regression	Linear regression
	OR (95% CI)	β (95% CI)
Virus infection		
Positive vs negative	6.4 (2.7, 15.3) [‡]	15.8 (11.2, 20.4) [‡]
Drinking ^a		
Yes vs No	–	10.6 (4.8, 16.3) [‡]
BMI (kg/m ²)		
≥ 25 vs < 25	5.7 (2.4, 13.7) [‡]	7.2 (2.7, 11.7) [‡]
VCM exposure		
High vs low	2.7 (0.8, 9.0)	1.2 (–4.3, 6.8)
GST T1 genotype		
Positive vs null	7.1 (2.1, 24.6) [‡]	4.9 (–0.8, 10.6)*
Interactions between VCM exposure and GST T1 genotype ^b	0.03 (0.004, 0.2) [‡]	–10.7 (–18.3, –3.1) [‡]
CYP2E1 · genotype		
c2c2 vs c1c1/c1c2	0.5 (0.04, 5.7)	–5.9 (–18.5, 6.7)
Interactions between VCM exposure and CYP2E1 genotype ^c	17.2 (0.8, 350.0)**	18.2 (1.1, 35.3)***

* $P = 0.09$; ** $P = 0.06$; *** $0.01 < P < 0.05$; [‡] $P < 0.01$; other P -value > 0.1

^aDrinking, Habitual alcohol drinking $\geq$ once a week

^bInteractions between VCM exposure and GST T1 genotype were identified in the logistic regression (see the text)

^cInteractions between VCM exposure and CYP2E1 genotype were identified in the logistic regression (see the text)

OR for individuals with combined GST T1 positive and high VCM exposure differed by only 0.6 times (OR of $2.7 \times 7.1 \times 0.03 \approx 0.6$, 95% CI 0.1–2.2, $P > 0.1$). The OR of the CYP2E1 c2c2 genotype on abnormal ALT was highly significant in the high VCM exposure group (OR of $2.7 \times 0.5 \times 17.2 \approx 23.3$, $P < 0.01$, 95% CI 3.1–176.1), but was insignificant in the low VCM exposure group (OR 0.5, $P > 0.1$, 95% CI 0.0–5.7). Similar interactions were also observed with the linear regression model (Table 4).

Discussion

In this study population, the frequency of the CYP2E1 c2 allele of 24.9%, is comparable to that shown in the previous study for Taiwanese (Stephens et al. 1994; Chao et al. 1995). The frequency of the GST M1 null genotype in our present study group (60.6%) is similar to that in the control group of a hepatocellular carcinoma study in Taiwan (63.3%; Yu et al. 1995). The frequency of GST T1 in Taiwan, however, has not been reported. The frequency of the GST T1 null genotype in China is 64.4% (Nelson et al. 1995) differing from our result of 47.4%.

Virus infection and BMI of $\geq 25 \text{ kg/m}^2$ are shown to be the major risk factors for abnormal ALT in this study. Habitual alcohol drinking is weakly associated with abnormal ALT because alcohol may selectively inhibit ALT activity (Redlich and Brodtkin 1994). In stratified analyses, the positive GST T1 shows a different effect on inducing abnormal ALT at different levels of VCM exposure, and CYP2E1 c2c2 is shown to be associated with abnormal ALT only in the high-VCM exposure group. Similar interactions between the VCM exposure and the genotypes of GST T1 and CYP2E1 are seen when analysed with the multiple logistic as well as the linear regression model.

In the present study, the VCM exposure and the genotypes of GST T1 and CYP2E1 are shown to interact with each other to affect ALT. Since these variables do not operate independently, the discussion of the individual effect of VCM exposure and the genotypes of GST T1 and CYP2E1 on the induction of liver damage should be avoided. The OR for individuals with positive GST T1 at high VCM exposure on abnormal ALT is 0.6 times the individuals with null GST T1 at low VCM exposure. However, the OR for individuals with positive GST T1 at low VCM exposure on abnormal ALT was 7.1 times higher than individuals with null GST T1 at low VCM exposure. Thus, the effect of GST T1 on inducing liver damage differed with different levels of VCM exposure.

The role of newly discovered GST T1 remains controversial. Different substrates metabolized by GST T1 may have different effects (Hallier et al. 1993; Norppa et al. 1995; Thier et al. 1993; Nelson et al. 1995). The metabolic pathway for VCM may depend on its concentration. At a low concentration, VCM may be pre-

dominantly metabolized by alcohol dehydrogenase to form 2-chloroethanol, CAA and 2-chloroacetic acid. At a high concentration, it may be oxidized to form CEO by cytochrome P-450s and/or spontaneously rearranged to form CAA (Easter and Von Burg 1994). The above alkylating metabolites including CAA and CEO may be further metabolized by the GSTs (Bolt 1986; Easter and Von Burg 1994). Thus, individuals with positive GST T1 exposed to different levels of VCM may have different effects on liver function due to different phase I metabolites formed. Furthermore, VCM may be directly metabolized by GSTs to conjugate with GSH (Hefner et al. 1975) and these GSH-conjugates may have mutagenic activity, such as ethylene dibromide conjugated with GSH to form alkylating intermediates (Thier et al. 1993). The GST T1 positive genotype is significantly associated with increased OR on abnormal ALT only at low VCM exposure as observed in this study. Therefore, VCM may be mainly metabolized directly by GST T1 at low concentration to form more toxic intermediates.

The transcriptional activity of the CYP2E1 c2c2 genotype has been shown to be about 10 times the activity of the c1c1 genotype (Hayashi et al. 1991); however, the level of the final protein product has not been verified. VCM is primarily metabolized by CYP2E1 at high concentration, in partial explanation of why the significant association of the CYP2E1 c2c2 genotype with increased OR on abnormal ALT only at high VCM exposure.

The above observation, therefore, indicates that GST T1 and CYP2E1 may play important roles in the biotransformation of VCM; hence, at low concentration, VCM is metabolized by GST T1 or alcohol dehydrogenase and at high concentration, by CYP 2E1. Accordingly, the effect of these metabolic enzymes on the induction of abnormal ALT is different at different levels of VCM exposure. The association between abnormal ALT and the polymorphism of alcohol dehydrogenase among these PVC workers should be investigated further to elucidate the roles of metabolic traits on the toxic effects of VCM.

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