A) FINAL PROGRESS REPORT

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List of Terms and Abbreviations:

VC, vinyl chloride; ASL, angiosarcoma of the liver; CYP2E1, cytochrome P450 2E1; CEO, chloroethylene oxide; CAA, chloroacetaldehyde; XRCC1, x-ray cross complementing-1 protein; XPD, xeroderma pigmentosum D protein

Abstract

One of the NIOSH's primary research objectives is "the identification and investigation of the relationships between hazardous working conditions and associated occupational diseases". A critical part of this process is understanding the complex relationship between workers' genetic make-up and workplace exposures, because it is becoming increasingly important in identifying workers at the greatest risk for developing occupational diseases and for providing possible interventions to reduce that risk and prevent disease. A potential model population for the study of such occupational gene-environment interactions and for the development of related interventions is provided by workers exposed to the carcinogen vinyl chloride (VC), because they represent an important segment of the petrochemical industry workforce and because much is known about the carcinogenic pathways for VC that could account for different health risks in similarly exposed workers. The purpose of this research has been to identify specific biomarkers of acquired and inherited genetic risk in VC-exposed workers that account for different cancer outcomes.

This research has identified several important acquired and inherited genetic risk factors for VC-induced cancer. The acquired genetic risks were identified as specific mutant biomarkers in VC-exposed workers' blood samples in the proteins of a major oncogene and a major tumor suppressor gene that correlated with the genetic changes in the resultant tumors. These biomarkers were found to occur in a highly statistically significant dose-response relationship with regard to estimated cumulative VC exposure. Furthermore, these biomarkers have been identified not only in VC-exposed workers with cancers but also in VC-exposed workers without any apparent neoplastic disease, even in workers exposed only below the current permissible exposure limit. However, at any given VC exposure level, some workers will have none, one or both mutant biomarkers. This individual variability was found to be due to inherited genetic differences in the proteins that metabolize VC or repair the mutagenic damage it produces. These biomarkers of inherited genetic risk in metabolism and repair were found to be statistically significantly associated with the occurrence of the biomarkers of acquired genetic risk regardless of the level of VC exposure.

These results can be utilized in the workplace for improvement of both primary and secondary prevention of VC-related cancer. For example, the fact that the mutant biomarkers of acquired genetic risk are statistically elevated even in workers exposed only below the current permissible exposure limit suggests that the current limit is not adequately protective. In fact, a new risk assessment could be based on the presence of these biomarkers, since exposures below one-quarter of the current permissible exposure limit demonstrate no statistically significant increase in these biomarkers. Furthermore, individuals with either the acquired or inherited biomarkers who are clearly at increased risk for the development of cancer could be targeted for specific secondary preventive interventions designed to address their particular genetic defect and avoid the occurrence of cancer. Such studies are currently underway for both the acquired and inherited genetic risks. Finally, it should be emphasized that although these studies have been focused on VC-exposed workers, this model should have much broader implications, since many other workplace chemical exposures are metabolized and repaired by the same pathways (and thus would be affected by the same inherited genetic susceptibilities) and results in similar genetic mutations (i.e., similar acquired genetic susceptibilities) and thus would be suitable targets for the same types of preventive approaches, so these results could be relevant for many other workers.

Section 1

Highlights/Significant Findings:

This research has a number of significant findings. It has identified several important acquired and inherited genetic risk factors for occupational cancer caused by vinyl chloride (VC) exposure. The acquired genetic risks were identified as specific mutant biomarkers in the blood of VC-exposed workers. These biomarkers are the protein products of two major cancer-related genes (an oncogene and a tumor suppressor gene), and they are correlated with the genetic changes in the resultant VC-induced cancers. These biomarkers were found to occur in a highly statistically significant dose-response relationship with regard to estimated cumulative VC exposure. Furthermore, these biomarkers have been identified not only in VC-exposed workers with cancers but also in VC-exposed workers without any apparent neoplastic disease, even in workers exposed only below the current permissible exposure limit. At any given VC exposure level, some workers will have none, one or both mutant biomarkers. This individual variability was found to be due to inherited genetic differences (genetic polymorphisms) in the proteins that metabolize VC or repair the genetic damage it produces. These biomarkers of inherited genetic risk in metabolism and repair were found to be statistically significantly associated with the occurrence of the biomarkers of acquired genetic risk regardless of the level of VC exposure.

Translation of Findings:

The findings of this research can be used to prevent occupational cancers related to VC exposure in several ways. First, they can be used to improve primary prevention of these cancers. As noted above, the biomarkers of acquired genetic risk occurred in a highly statistically significant dose-response relationship with regard to estimated cumulative VC exposure, even in workers exposed only below the current permissible exposure limit. This suggests that the current permissible exposure limit may not be adequately protective. However, these biomarkers could be used to refine the risk assessment that forms the basis for the current limits to make it more protective. For example, although workers with VC exposures between one-quarter of the current limit and the current limit also had a statistically significant increase in the rate of occurrence of these biomarkers of acquired genetic risk (actually at a rate that was statistically similar to that of workers exposed above the current limit), workers exposed only below one-quarter of the current limit did not have a statistically significant increase in these biomarkers. Thus, an exposure limit at or below one-quarter of the current limit might provide significantly more protective. These biomarkers can also be used to improve secondary prevention of VC-related cancers. Among VC workers at any exposure level, not all will be at risk for cancer. These biomarkers can presumably identify those workers who are the greatest risk of cancer, and they can then be targeted for more aggressive interventions to prevent the occurrence of cancer. Specifically, the interventions can be targeted to the exact genetic defects identified by each particular biomarker.

Outcomes/Relevance/Impact:

The results of this research have several significant potential outcomes which could impact workplace risk if used, as suggested above. The results could lead to better primary prevention of occupational cancer by allowing refinement of the risk assessment that forms the basis for the permissible exposure limit for VC. The results could also lead to better secondary

prevention of occupational cancer by identifying exposed workers at the highest risk for subsequent development of cancer who could be targeted for more aggressive interventions directed at the specific genetic defects indicated by the biomarkers.

Section 2

Scientific Report

In all of this research over the past nine years, the overall focus has been to identify the inherited and acquired genetic defects associated with an increased risk of cancer from VC exposure. This has been investigated using two complementary approaches – epidemiologic investigation of genetic biomarkers in VC exposed workers and laboratory investigation of biological samples from VC exposed workers under controlled experimental conditions to provide the biological plausibility to support the epidemiologic findings.

The background rationale for this particular study is provided by the general idea that a critical part of understanding occupational carcinogenesis depends on elucidating the complex relationship between workers' genetic make-up and workplace exposures, and a VC-exposed cohort represents a potential model population for studying this relationship because much is known about VC carcinogenic pathways. VC is a known animal and human carcinogen associated with the sentinel neoplasm of angiosarcoma of the liver (ASL), as well as other malignancies and non-cancer health effects, and used in large quantities around the world primarily in the manufacture of polyvinyl chloride polymers. VC is a gas, and following inhalation exposure, absorption is rapid in humans. VC then undergoes phase I metabolism primarily in the liver by cytochrome P450 2E1 (CYP2E1), and the resultant electrophilic metabolites chloroethylene oxide (CEO) and chloroacetaldehyde (CAA) are further metabolized in phase II reactions by glutathione-S-transferases (GSTs) and aldehyde dehydrogenase 2 (ALDH2) to end products for excretion. However, CEO and CAA are known to be able to induce DNA damage in the parenchymal liver cells, as well as the non-parenchymal endothelial cells, and are believed to be the proximate carcinogens in the VC carcinogenic process. Both CEO and CAA are capable of forming etheno-DNA adducts, three of which have been identified *in vivo*: N^2,3-εG; 1,N^6-εA; and 3,N^4-εC. The εG and εA adducts are pro-mutagenic and account for the production of specific point mutations (G->A transitions and A->T transversions, respectively) in cellular oncogenes and tumor suppressor genes (Ki-*ras* and *TP53*, respectively) in VC-associated ASLs from exposed workers.

Thus, initially the research focused on the use of the protein products of these mutant genes (mutant *ras*-p21 and mutant p53) as acquired genetic biomarkers of effect from VC exposure that might be used to identify workers at risk for the subsequent development of cancer. First, we found that the immunological detection of circulating mutant *ras*-p21 protein or of mutant p53 protein (or antibodies to mutant p53 protein) could serve as serum biomarkers for the occurrence of the corresponding VC-induced carcinogenic mutations in the target tissue of exposed individuals. These serum biomarkers were identified not only in VC-exposed workers with ASLs but also in VC-exposed workers without any apparent neoplastic disease, even in workers exposed only below the current permissible exposure limit. The presence of these biomarkers was found to occur with a highly statistically significant dose-response relationship with regard to estimated, cumulative VC exposure, suggesting that the generation of the biomarkers was indeed a result of the exposure. However, at any given VC exposure level, some workers would have none, one or both mutant biomarkers. It was hypothesized that one possible explanation for this inter-individual variability was inherited genetic differences in the enzymes that metabolize VC or repair the mutagenic damage it produces.

Over the course of subsequent funding periods of this grant, we have been able to successfully identify and verify some of the key genetic polymorphisms responsible for this inter-individual variability (as well as eliminating several other possibilities as not significantly

contributory, such as GSTs and ALDH2). For example, we identified the *CYP2E1 c2* allele as the major contributor to genetic variability in the metabolism of VC, since it is statistically significantly associated with an increased occurrence of the mutant *ras*-p21 and mutant p53 biomarkers even after controlling for potential confounders including cumulative VC exposure. These epidemiologic results were consistent with the fact that the *c2* variant CYP2E1 was demonstrated to have higher expression levels (and thus higher activity) than the normal CYP2E1 allele and therefore generates more reactive intermediates CEO and CAA and more etheno-DNA adducts at any given level of VC exposure. Furthermore, we have similarly identified genetic polymorphisms in two DNA repair pathways that also contribute significantly to the inherited variability in response to VC exposure.

The types of etheno-DNA adducts induced by VC would normally be expected to be corrected by base excision repair (BER), a process which is coordinated by the x-ray cross complementing-1 (XRCC1) protein. Several common inherited genetic polymorphisms have been identified in *XRCC1*, the most common being at codon 399. We have shown that the codon 399 *XRCC1* polymorphism is a major contributor to genetic variability in the repair of one type of DNA damage from VC, namely the εA adducts, since it is statistically significantly associated with an increased occurrence of the mutant p53 biomarkers, even after controlling for potential confounders including cumulative VC exposure, with a potentially supra-multiplicative gene-environment interaction between the polymorphism and VC exposure. These epidemiologic observations are consistent with several experimental findings including the fact that this polymorphism produces a significant conformational change in the XRCC1 protein that could disrupt its interaction with other components of the BER repair machinery affecting their activity, which results in an increased level of the εA adducts in cells at any given level of VC exposure, and these adducts are then responsible for the mutations in the *p53* gene and thus the mutant p53 biomarkers. However, we were not able to demonstrate a statistically significant association between the *XRCC1* polymorphism and the occurrence of the other biomarker for mutant *ras*-p21. Therefore, most recently we examined polymorphisms in other DNA repair pathways to try to account for the genetic variability in the occurrence of the mutant *ras*-p21 biomarker.

Another major mechanism for DNA repair is nucleotide excision repair (NER) which is dependent on the xeroderma pigmentosum (XPD protein to act as a DNA helicase to promote bubble formation at the site of DNA damage to allow repair to occur. Two common polymorphisms have been identified in XPD at amino acid residues 312 and 751. When we examined the effect of these *XPD* polymorphisms on the VC-associated mutant biomarkers we found highly statistically significant effects for the *XPD* 312 and 751 polymorphisms on the occurrence of the mutant *ras*-p21 biomarker, even after controlling for potential confounders including cumulative VC exposure, with a potentially supra-multiplicative gene-environment interaction between the 751 polymorphism and VC exposure and a potentially supra-multiplicative gene-gene interaction between the *XPD* polymorphisms and the *CYP2E1* polymorphism. Once again, these epidemiologic results were found to be consistent with several preliminary experimental findings including the fact that the 751 polymorphism produces a significant conformational change in the XPD protein that could disrupt its interaction with its helicase activating protein, which results in an increased level of the εG adducts in cells at any given level of VC exposure, and these adducts are then responsible for the mutations in the *ras* gene and thus the mutant *ras*-p21 biomarkers.

In conclusion, as originally proposed through epidemiologic observations and experimental results, we have been able to construct a relatively complete picture of the VC carcinogenic pathway that accounts for the major inherited and acquired genetic defects

associated with an increased risk of cancer from VC exposure. These results have several significant implications for future applications to reduce this risk though both primary and secondary prevention. As noted, the biomarkers of acquired genetic risk for mutant p53 and mutant *ras*-p21 were found to occur in a highly statistically significant dose-response relationship with regard to estimated cumulative VC exposure, even in workers exposed only below the current permissible exposure limit. This suggests that the current permissible exposure limit may not be adequately protective. However, these biomarkers could be used to refine the risk assessment that forms the basis for the current limits to make it more protective. For example, although workers with VC exposures between one-quarter of the current limit and the current limit also had a statistically significant increase in the rate of occurrence of these biomarkers of acquired genetic risk (actually at a rate that was statistically similar to that of workers exposed above the current limit), workers exposed only below one-quarter of the current limit did not have a statistically significant increase in these biomarkers. Thus, an exposure limit at or below one-quarter of the current limit might provide significantly more protective. Biomarkers of both inherited and acquired genetic defects that indicate increased cancer risk can also be used to improve secondary prevention of VC-related cancers. Among VC workers at any exposure level, not all will be at risk for cancer. These biomarkers can presumably identify those workers who are the greatest risk of cancer, and they can then be targeted for more aggressive interventions to prevent the occurrence of cancer. Specifically, the interventions can be targeted to the exact genetic defects identified by each particular biomarker. For example, in individuals with increased metabolism of VC to its reactive intermediates (e.g., with the inherited *c2 CYP2E1* allele), the activity of CYP2E1 can be decreased through simple dietary interventions (e.g., watercress) which should decrease the generation of adducts and thus the risk of cancer. In individuals with the acquired genetic mutations (e.g., with the mutant p53 biomarker), the activity of normal p53 can be restored (e.g., with specially designed p53 peptides) resulting in apoptosis in the damaged cells before they can become cancers. These types of chemoprophylactic interventions for secondary prevention are currently under study. Finally, it should be noted that these results are relevant not only for improving the health of workers exposed to VC but also for workers in a range of industries. Many other workplace chemicals are metabolized by the same pathways and/or produce DNA damage repaired by the same pathways and result in similar DNA mutations as VC (and thus would be affected by the same inherited and acquired genetic defects and would be suitable targets for the same preventive interventions), so the current findings could have much broader applicability and impact.

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Inclusion of Gender and Minority Subjects

This research involved French and Chinese VC workers. Due to the nature of these workforces, which historically did not include women or other minorities, women and other minorities could not be included in this study.

Inclusion of Children

No children were not included in this study due to the nature of the populations from which the subjects were drawn, namely adult workers.

Materials Available for Other Investigators

Not Applicable.