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Plastics and carcinogenesis: The example of vinyl chloride

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Abstract

The manufacture, use and disposal of various plastics can pose numerous health risks, including the risk of cancer. A model example of carcinogenic risk from plastics is provided by polyvinyl chloride, since it is composed of the known human carcinogen vinyl chloride (VC). In recent years, much has been learned about the molecular biological pathways of VC carcinogenesis. This has led to molecular epidemiologic studies of VC carcinogenesis in exposed human populations which have identified useful biomarkers of exposure, effect and susceptibility for VC. These studies have in turn provided the basis for new molecular approaches for the prevention and treatment of VC cancers. This model could have much wider applicability for many other carcinogenic exposures and many other human cancers.

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Full Text

Introduction

Polyvinyl chloride (PVC) is one of the most commonly manufactured plastics in the world, used in a wide variety of products including packaging, pipes, automotive parts, construction materials and furniture. PVC is polymerized from vinyl chloride (VC) monomer, which is one of the highest production volume chemicals globally with a current annual worldwide demand of approximately 16 billion pounds which is increasing at an approximate 3% annual rate. Up to 98% of VC is used in the production of PVC. [1]

Unfortunately, VC is a well-established animal and human carcinogen. It is most strongly associated with liver cancer, in particular the rare, sentinel neoplasm of liver angiosarcoma (LAS), a malignant tumor of the endothelial cells of the liver. [1] However, VC has also been identified as a cause of hepatocellular carcinoma (HCC), the corresponding malignant tumor of the parenchymal cells of the liver. [2] It has also been associated with non-malignant health effects, including in the liver and other organs, as well as other malignancies (e.g., lung and brain), although these other carcinogenic associations remain much more controversial. The most significant exposures to VC occur in the petrochemical and plastics' industries. For example, national institute for occupational safety and health (NIOSH) has estimated that 81,000 workers employed in more than 3,700 worksites are potentially exposed to VC in the US; worldwide estimates are much higher with more than 2,200,000 workers probably exposed to VC. General population exposures also occur primarily through the air and water. For example, elevated levels of VC have been found not only in the air near VC manufacturing and processing facilities but also in the vicinity of many hazardous waste sites and municipal landfills, either due to the direct disposal of VC or from the microbial degradation of other chlorinated solvents to form VC. In some cases, dangerously high levels have been detected (up to 44 ppm; compared to a US Environmental Protection Agency Reference Concentration of 0.04 ppm) in the air at some of these landfills. [1] General population exposures may also occur from tobacco smoke, drinking water from PVC pipe, and food and beverages from PVC packaging and bottles, although probably at much lower levels. However, these are some of the reasons that authorities in the field of chemical safety have warned that VC is very much "still a cause of concern" in occupational and environmental health. [3] Within the plastics' industry, VC is an also an excellent model for the study of chemical carcinogenesis via genotoxic mechanisms because it is a well-known DNA-reactive chemical, for which much has been learned about the molecular biology of its pathways of action[Figure 1]. [4] It should be noted that although the mutagenic pathways for VC carcinogenesis have been the best studied and are thus the focus here, it is quite possible that other mechanisms could contribute significantly to VC carcinogenesis through more indirect pathways, including, for example, through epigenetic dysregulation of gene expression or alterations in immune surveillance.{Figure 1}

The Molecular Biology of Vinyl Chloride Carcinogenesis

VC is a gas so the most significant exposures are respiratory. Following inhalation exposure, absorption is rapid in humans and most subsequent metabolism occurs in the liver. [1] Phase I metabolism is primarily via the cytochrome P-450 isoenzyme 2E1 (CYP2E1) to generate the reactive intermediates chloroethylene oxide (CEO) and chloracetaldehyde (CAA) which are further metabolized in Phase II reactions by glutathione-S-transferases (GSTs) and aldehyde dehydrogenase 2 (ALDH2) to end-products for ultimate excretion. However, CEO and CAA can readily interact with cellular macromolecules, including DNA, to produce promutagenic effects. Because CEO tends to react much more rapidly with nucleic acids than CAA, it is usually regarded as the most relevant electrophile for the generation of DNA adducts and consequent mutagenic effects; the greater biological relevance of CEO is also supported by comparisons of the adduct profile of VC with that of 2,2-dichloroethyl ether, which only produces CAA as a metabolite.VC biotransformation to CEO probably occurs principally in hepatocytes, but the epoxide can also reach and react with adjacent sinusoidal lining cells, so that mutagenic effects can occur in parenchymal liver cells and non-parenchymal endothelial cells, providing a logical rationale for the association between VC exposure and LAS as well as HCC. [5] The major VC-associated liver DNA adduct is 7-(2-oxoethyl)guanine, comprising up to 98% of all adducts formed. However, this adduct is eliminated from the DNA with a very short half-life, principally by chemical depurination, and is not considered to be promutagenic. On the other hand, three etheno DNA adducts are also

formed in much less abundance, but they are known to be promutagenic. These are: N 2 ,3-ethenoguanine (εG); 1,N 6 -ethenoadenine (εA); and 3,N 4 -ethenocytosine (εC). [6] It should be noted that these etheno-DNA adducts can also be found in tissues from unexposed humans and animals because they can be produced endogenously through the interaction of lipid peroxidation-derived aldehydes and hydroxyalkenals. However, VC exposure can increase the level of these adducts 10-100 fold over background in the hepatocytes and non-parenchymal liver cells of exposed animals. [5]

There are several potential mechanisms by which the VC-induced adducts could be repaired before they have a chance to cause mutations. As noted above, the oxoethyl adduct is removed rapidly by chemical depurination. The potential repair of the etheno adducts is more complicated. The 1,N 6 -εA adducts are recognized and removed by 3-methyl adenine DNA glycosylase which is part of the base excision repair (BER) pathway; this is accomplished by hydrolyzing the N-glycosidic bond between the damaged base and deoxyribose, leaving an abasic site in the DNA. [5] The BER apparatus includes numerous other proteins that complete the repair at the abasic site once the adduct is removed. The X-ray cross-complementing-1 (XRCC1) protein is critical to this process since it acts as a scaffold protein in this pathway and appears to regulate and/or enhance the activity of other BER proteins, which include an apurinic/apyrimidinic endonuclease (APE1), poly (ADP-ribose) polymerase-1 (PARP-1), poly (ADP-ribose) polymerase-2 (PARP-2), DNA polymerase β (Pol β) and DNA ligase IIIα (Lig III). AP endonuclease is responsible for cleaving the phosphodiester bond at the abasic site created by the glycosylase. PARP-1 and to a lesser extent PARP-2 participate in the repair process by catalyzing ribosylation of a number of DNA-bound proteins, thereby decreasing the affinity of these proteins for DNA, and allowing the repair machinery to access the damaged site. PARP-1 and PARP-2 can homo- and hetero-dimerize and interact with Pol β, Lig III and XRCC1. Pol β, the polymerase involved in short patch repair, provides two essential activities, deoxyribophosphodiesterase activity which releases the 5' sugar phosphate group, and gap-filling synthesis, where one nucleotide is added to the 3' OH. Finally, Lig III seals the nick in an ATP-dependent manner. [7],[8] Although XRCC1 has not been demonstrated to contain enzymatic activity of its own, it is necessary for coordinating and regulating the early and late stages of BER through protein interaction modules such as the BRCA1 Carboxy Terminus (BRCT) domains. [9],[10] As discussed below, alterations in any of these proteins, particularly XRCC1, that could affect the efficiency of BER might be expected to result in an increase in the εA adduct levels at any given level of VC exposure. The 3,N 4 -ethenocytosine adduct is also repaired with high efficiency by BER via the thymine DNA glycosylase. As a result, both the εA and εC adducts have reasonably short half-lives in the range of one day. [6]

In contrast, the N 2 ,3-ethenoguanine adduct has been shown to have a considerably longer half-life, in the range of 150 days, suggesting that it is not very efficiently repaired. [6] This is consistent with the results of in vitro studies that indicate that etheno-guanine adducts are poorly repaired by BER. [11] Thus, if they are repaired at all, it is likely to be by a different DNA repair pathway. Another major mechanism for DNA repair is nucleotide excision repair (NER). Like BER, NER occurs in a series of steps: damage recognition, unwinding and demarcation of the DNA, excision of the single-stranded fragment containing the damaged site, and DNA re-synthesis. NER is accomplished primarily through the action of proteins of the xerodermapigmentosum family of genes which are categorized into seven different groups (A-G). XPC and XPE proteins are involved in recognition of different types of DNA damage. XPB and XPD are DNA helicases that function as subunits of the transcription factor IIH complex (TFIIH) to promote DNA bubble formation at the damaged site by unwinding the DNA as XPA complexes with RPA proteins for demarcation. XPF and XPG are structure-specific endonucleases for excision of the damaged site. Finally, replicative DNA polymerase and DNA ligase I complete the repair. [12],[13] XPD is one of the major players in NER and is essential for life. [14],[15] As discussed below, alterations in any of these proteins, particularly XPD, that could affect the efficiency of NER might be expected to increase the εG adduct levels at any given level of VC exposure.

The promutagenic properties of etheno-DNA adducts that are not fully repaired by one or another of the DNA repair pathways have been well-documented in experimental systems in vitro, as well as in vivo in bacterial and mammalian cells. The εG adduct generates G->A base changes; the εA adduct generates A->G, A->T and A->C base changes; and the εC adduct generates C->A and C->T base changes. [5] These experimental results are consistent with the tumor mutational spectra identified in exposed animals and humans in oncogenes and tumor suppressor genes, although there are both important similarities and differences between the patterns seen in the animal tumors and the

human tumors. Both HCCs and ASLs in VC-exposed rats were found to have mainly A->T transversions in the cancer-related H-ras and p53 genes. [5] For example, seven of eight VC-induced HCCs in VC-exposed rats had an A->T transversion at Codon 61 of H-ras; [16] 11 of 25 ASLs and one of eight HCCs in VC-exposed rats had mutations in p53, all but one of which were base pair substitutions with nine at A:T base pairs (5 A->T, 2 A->G, 2 A->C) and three at G:C base pairs (all G->A), and two of the A->T transversions occurred at the same site (the first nucleotide of Codon 253). [17] On the other hand, ASLs in VC-exposed humans have been found to have exclusively A->T transversions in p53 (three of six, including one at the first nucleotide of Codon 255 which corresponds to the same mutation in Codon 253 seen in rats). [18] Of note, studies of p53 mutations in 21 human ASLs that were not associated with VC exposure, only two had mutations , neither of which were A->T transversions. [19] Studies of VC-associated HCCs have also found frequent p53 mutations, occurring in 11 of 18 cases, although only two of these were A->T transversions. [20] Both ASLs and HCCs in VC-exposed humans have been found to have primarily G->A transitions in the K-ras oncogene at Codons 12 and 13. For example, in studies of VC-associated HCCs, 5 of 12 tumors were found to have K-ras mutations at Codons 12 and 13, three of which were G->A transitions. [21] Even more striking, in studies of K-ras gene mutations in VC-associated ASLs, G->A transitions were found in 23 of 33 tumors with the vast majority of these (17 of 23) occurring at Codon 13. [22],[23] Again of note, studies of K-ras mutations in 24 ASLs that were not associated with VC exposure did find G->A transitions in seven cases, but all of them were at Codon 12, indicating that the Codon 13 mutation may be relatively specific for VC-induced ASLs. [24] Interestingly, in studies of both ASLs and HCCs in VC-exposed humans, five cases were also found to contain K-ras mutations in the histologically normal adjacent tissue, three of which were G->A transitions and two of which occurred at Codon 13; [21],[22] likewise, at least one case of a non-dysplastic pre-angiosarcomatous liver lesion has been found to contain the characteristic G->A transition at Codon 13 of K-ras. [25] These results suggest that these VC-associated mutations, particularly the Codon 13 K-ras mutation, may be a relatively early event in VC carcinogenesis, and thus the occurrence of these mutations may be useful biomarkers of cancer risk in exposed individuals, as discussed below.

The G->A transition at Codon 13 of K-ras results in the substitution of an aspartic acid (Asp) for the normal glycine (Gly) at amino acid residue 13 in the encoded p21 protein product. This substitution is believed to be oncogenic, having been identified in other human tumors as well. The oncogenic mechanism of action of this substitution is thought to be through the production of a conformational change in p21 which may be responsible for altering its intrinsic GTPase activity, thus affecting signal transduction within the cell leading to uncontrolled growth and division. [4] Similarly, the A->T transversions at various codons of p53 produce their corresponding amino acid substitutions in the encoded p53 protein product, all changes that have been shown to cause the protein to adopt its so-called "malignant" conformation with a concomitant loss of its normal tumor suppressor activity. [4] These protein changes provide a useful indicator of the pathogenic consequences of the occurrence of the corresponding mutations as well as convenient intermediate biomarkers of the VC effect to study the molecular epidemiology of VC carcinogenesis in exposed human populations.

The Molecular Epidemiology of Vinyl Chloride Carcinogenesis

It has been shown that the mutant ras-p21 protein containing Asp for Gly at amino acid residue 13 can be distinguished from the wild-type protein and other mutant ras-p21 proteins immunologically with a mouse monoclonal antibody specific for this protein. For cells in culture that contain the mutant ras gene, it is possible to use this monoclonal antibody to detect mutant ras-p21 expression in the cells by immunocytochemistry and in the extracellular supernatant by immunoblotting. In analogous situations in vivo, mutant Asp 13 ras-p21 can be detected in tumor tissue by immunohistochemistry and in the serum by immunoblotting of VC-exposed workers with ASLs known to contain the mutant ras gene but not in the serum of VC-exposed workers with ASLs that do not contain the mutation or in unexposed controls. [4],[26],[27]

An analogous, although slightly more complicated situation occurs with p53. As noted, all of the VC-induced mutations in the p53 gene have been shown to cause a similar conformational change in the encoded p53 proteins that result in

the exposure of a common epitope, which is normally not immunologically detectable in the wild-type protein. Thus, these mutant p53 proteins can be distinguished from wild-type p53 immunologically with a mouse monoclonal antibody that binds to this mutant-specific epitope. For cells in culture that contain the mutant p53 genes, it is possible to use this monoclonal antibody to detect mutant p53 protein expression in the cells by immunocytochemistry and in the extracellular supernatant by immunoblotting or by enzyme-linked immunosorbent assay (ELISA). In the analogous situation in vivo, mutant p53 can be detected in the tumor tissue by immunohistochemistry and in the serum by immunoblotting or ELISA of VC-exposed workers with ASLs known to contain the mutant p53 genes but not of VC-exposed workers with ASLs that do not contain the mutations or in unexposed controls. In some cases of mutant p53-positive tumors, it is known that individuals can also develop an antibody response to the mutant p53 which can obscure the detection of the mutant p53 protein itself. However, it is also possible to detect these auto-antibodies to mutant p53 using an ELISA. Thus, the detection in serum of mutant p53 protein and/or an antibody response to mutant p53 protein can be used together to best identify individuals who have a p53 mutation in their tumors. [4],[28],[29]

Based on the above evidence, it seems that these serum biomarkers for mutant ras-p21 and mutant p53 accurately reflect the occurrence of the corresponding mutational changes in the target tissue of VC-exposed workers. Further support for this is provided by the case of an ASL with multiple serum samples over time for which the levels of these serum biomarkers seemed to parallel the clinical course of the disease in terms of tumor burden. [4],[23] In addition, these biomarkers have been identified not only in VC-exposed workers with ASLs but also in VC-exposed workers with non-malignant (but potentially pre-malignant) angiomatous lesions and in VC-exposed workers without any apparent neoplastic disease, even in workers exposed below the current permissible exposure limit of 1 ppm. [4],[30],[31],[32] In a large cohort of French VC workers, the presence of these biomarkers was found to occur with a highly statistically significant dose-response relationship with regard to estimated, cumulative VC exposure, supporting the claim that the generation of the biomarkers was indeed the result of the exposure. [33] Similar results with these biomarkers have been noted in several other VC workers' cohorts around the world. [34],[35],[36],[37],[38],[39] To date in these various studies, at least five VC-exposed biomarker-positive workers without ASL have developed subsequent liver lesions presumed or confirmed to be ASL, also suggesting that these biomarkers may have predictive value for the subsequent occurrence of cancer.

However, at any given level of VC exposure, some workers will have none, one or both mutant biomarkers. One possible explanation for this inter-individual variability is genetic differences in the proteins that metabolize VC or repair the DNA damage it produces. For example, in the aforementioned French VC worker cohort we have identified the CYP2E1 c2 allele as a significant contributor to genetic variability in the metabolism of VC, since it is statistically significantly associated with an increased occurrence of either or both of the mutant ras-p21 and mutant p53 biomarkers even after controlling for potential confounders including cumulative VC exposure, and the gene-environment interaction between the polymorphism and VC exposure was approximately additive. [33],[40] Studies in other VC worker populations have found similar effects of the CYP2E1 polymorphism on these biomarkers, as well as other biomarkers of DNA damage such as micronuclei and sister chromatid exchanges or non-specific liver damage. [41],[42],[43],[44],[45],[46],[47],[48],[49],[50],[51],[52] This is consistent with recent experimental results from studies of lymphoblasts from individuals of different genotypes exposed in vitro to VC. Cells with the c2c2 CYP2E1 genotype were found to have approximately 2.5 times higher gene expression than those with the wild-type c1c1 genotype [Figure 2], which resulted in an approximate 2.1-fold increase in etheno-DNA adduct generation in the polymorphic cells compared to the normal cells at the same level of VC exposure [Table 1]. Other polymorphisms in the Phase II VC metabolic pathway including ALDH2, GSTM1 and GSTT1 have also been implicated in modulating VC-induced DNA damage in some but not all VC worker populations. [33],[40],[41],[42],[43],[44],[45],[46],[47],[50],[51],[52],[53],[54]{Figure 2}{Table 1}

As noted, another potential source of inter-individual variability in the susceptibility for VC-induced mutagenesis could derive from genetic differences in the DNA repair pathways for BER and NER. As described above, VC-induced εA and εC adducts would be expected to be repaired by the BER pathway, in which the XRCC1 protein plays the major role of coordinating the activity of the repair machinery. XRCC1 is known to contain three common polymorphic sites that might be expected to have an effect on XRCC1 structure and function because they occur in or near important

protein domains. [55] For example, the polymorphism at amino acid residue 194, which results in the substitution of a tryptophan for the normal arginine, occurs in the XRCC1 N-terminal domain from amino acid residues 1-195 that has been observed to mediate its interaction with the palm-thumb domain of Polβ. [56] A second polymorphism at amino acid residue 280, which results in the substitution of a histidine for the normal arginine, occurs in the region between the N-terminal domain and the BRCT1 domain of the protein and close to the nuclear localization signal site and thus could affect the relationship between these two critical domains and/or the protein's localization ability. [57] The third and most common polymorphism in XRCC1 occurs at amino acid residue 399, resulting in the substitution of a glutamine for the normal arginine, within the highly conserved central BRCT1 domain from amino acid residues 315-403, which has been associated with the functioning of PARP1, PARP2 and APE1. [58] In the aforementioned French VC worker cohort, we have been able to identify the effect of these XRCC1 polymorphisms on the occurrence of the mutant p53 biomarker, but not the mutant ras-p21 biomarker, even after controlling for potential confounders including cumulative VC exposure. [59],[60],[61] The difference in effect on the two biomarkers is expected, since, as noted the εA adducts that result in the mutant p53 biomarker are repaired by BER but the εG adducts that result in the mutant ras-p21 biomarker are not, so changes in XRCC1 might affect the former but should not affect the latter. Among these three XRCC1 polymorphisms, however, the most significant effect on the mutant p53 biomarker was attributable to the residue 399 polymorphism. In this case, individuals who were homozygous variant Gln-Gln at 399 had a statistically significant 1.9-fold risk of occurrence of the mutant p53 biomarker compared to homozygous Arg-Arg wild-type individuals, even after controlling for potential confounders including cumulative VC exposure, and the gene-environment interaction between the polymorphism and VC exposure appeared to be potentially supra-multiplicative. [61] Studies in other VC worker populations have found similar effects of the XRCC1 polymorphisms, particularly the 399 polymorphism, on the mutant p53 biomarker, as well as other biomarkers of DNA damage. [43],[48],[51],[62] This is consistent with various experimental results. For example, molecular modeling of the BRCT1 domains of the normal and polymorphic forms of XRCC1 demonstrates that the 399 substitution produces significant conformational changes in this domain, including the loss of secondary structural features such as α-helices that can be critical for mediating protein-protein interactions that would allow XRCC1 to coordinate BER. [63] Also, studies of lymphoblasts from individuals of different genotypes exposed in vitro to the reactive metabolites of VC showed that cells with the XRCC1 399 homozygous variant Gln-Gln genotype had an approximate fourfold decrease in efficiency of repair of εA DNA adducts compared to cells with the homozygous wild-type Arg-Arg genotype [Table 2], [60],[64] resulting in an approximate 1.8-fold increase in mutation frequency in the polymorphic cells as measured by the HPRT assay.{Table 2}

As discussed above, εG DNA adducts do not appear to be repaired well by BER and polymorphisms in XRCC1 do not appear to affect the occurrence of the mutant ras-p21 biomarker that results from the εG adducts in VC-exposed workers, so other DNA repair pathways may be involved. NER is another important DNA repair pathway that is critically dependent upon the XPD protein, which is also know to contain at least two common polymorphic sites, namely at amino acid residues 312 (Asp->Asn) and 751 (Lys->Gln). [65] The 751 site is assumed to be particularly important for XPD function since it occurs in the C-terminal domain of the protein which has been suggested to interact with the p44 helicase activator protein of the TFIIH complex; [66] also, it is been shown that an XPD mutation that results in the loss of the final 17 C-terminal amino acids, including residue 751, results in the clinical disease phenotype of trichothiodystrophy. [67] In the aforementioned French VC worker cohort, we have been able to identify the effect of these XPD polymorphisms on the occurrence of both mutant biomarkers, although the most marked and statistically significant effect was on the mutant ras-p21 biomarker. [61] In this case, individuals who were homozygous variant at either residue 312 or 751 had a statistically significant 2.6-3.0-fold increased risk of occurrence of the mutant ras-p21 biomarker compared to homozygous wild-type individuals, even after controlling for potential confounders including cumulative VC exposure. Furthermore, in the case of the residue 751 polymorphism, the gene-environment interaction between the polymorphism and VC exposure, as well as the gene-gene interaction between the XPD and CYP2E1 polymorphisms, appeared to be potentially multiplicative. [61] Once again, studies in other VC worker populations have found similar effects of the XPD polymorphisms on other biomarkers of DNA damage. [48] This is also consistent with various experimental results. For example, molecular modeling of the normal and polymorphic forms of XPD demonstrates that these substitutions produce discrete conformational changes in the protein which could affect its function [Figure 3]. [68] Also, studies of lymphoblasts from individuals of different genotypes exposed in vitro to the reactive metabolites of VC showed that cells with the XPD 751 homozygous variant

Gln-Gln genotype had an approximate fivefold decrease in efficiency of repair of εG DNA adducts compared to cells with the homozygous wild-type Lys-Lys genotype [Table 3]. Based on mutational spectrum studies in CAA-exposed human cell lines, [69] the resultant increase in εG DNA adducts would especially result in an increase in G->A transitions consistent with those found in the tumors of VC-exposed workers, as noted above.{Figure 3}{Table 3}

The Molecular Basis for Prevention and Treatment of Vinyl Chloride Carcinogenesis

A thorough understanding of the molecular biology and molecular epidemiology of VC carcinogenesis can provide the basis for new molecular approaches to the prevention and treatment of VC-induced cancers.

For example, the results of the molecular epidemiology of the mutant biomarkers could be used for improved primary prevention of VC-induced cancers by refining the risk assessment that is used as the basis for determining acceptably safe permissible exposure limits for VC. Since 1974 the permissible exposure limit for VC-exposed workers has been 1 ppm, as an 8-h time-weighted average, based on extrapolations from animal experiments. [1] This would be the equivalent of a maximum cumulative dose of 40 ppm-years over a 40-year working lifetime. Unfortunately, as noted above, we have found statistically significantly increased occurrences of the VC-induced mutant biomarkers even in workers exposed only below the permissible exposure limit of 1 ppm (i.e., with cumulative VC exposures less than 40 ppm-years). [30],[31] This might suggest that the current permissible exposure limit is not adequately protective against carcinogenic effects. However, cohorts of workers exposed to less than 40 ppm-years of cumulative VC exposure can be stratified into two sub-groups. Workers with 10-40 ppm-years of cumulative exposure have been found to have a statistically significantly increased occurrence of the mutant biomarkers at a rate which is actually not statistically different from workers with more than 40 ppm-years of cumulative VC exposure, whereas workers with less than 10 ppm-years of cumulative exposure did not have a statistically significantly increased occurrence of the mutant biomarkers compared to unexposed controls. [31] Thus, a risk assessment based on biomarkers might suggest a permissible exposure limit of 0.25 ppm as being more adequately protective of workers' health by preventing the occurrence of these cancer-related mutations.

In individual workers who are already exposed to VC at higher levels, there may be additional avenues for secondary prevention and treatment of VC-induced disease. For example, one approach to secondary prevention could be based on "personalized prevention" derived from knowledge of each worker's susceptibility from genetic and other factors. As noted above, the level of activity of CYP2E1 in Phase I metabolism of VC can have a significant impact on the amount of DNA damage produced at any given level of VC exposure. Many different individual factors can affect the level of expression and hence the level of activity of CYP2E1 including genetics (e.g., the c2 allele), alcohol consumption, diet (e.g., cruciferous vegetables) and medications (e.g., isoniazid). [70],[71] Although most of these factors increase the expression and activity of CYP2E1, certain vegetables act to decrease its activity. For instance, it has been demonstrated in controlled clinical studies in humans that ingestion of small amounts of watercress (Rorippa nasturtium-aquaticum) can effectively inhibit CYP2E1's metabolic activity. [72] One proposed mechanism for this effect is due to the fact that watercress is a rich source of glucosinolates, including gluconasturtiin, which is metabolized by intestinal microflora to phenyllethylisothiocyanate, a known CYP2E1 inhibitor. Cell culture studies have suggested an anticarcinogenic effect from isothiocyanate exposure, and some dietary studies in animals and humans have associated increased isothiocyanate consumption with decreased cancer risk. [73] Most significantly, a recent randomized, crossover, controlled clinical trial of low-dose watercress for a limited period of time was shown to statistically significantly decrease end-points of gross DNA damage (as measured in peripheral lymphocytes by the Comet assay) by 17%, with the effect being greatest in those individuals with exogenous carcinogen exposure (i.e., cigarette smokers). [74] These results suggest that watercress may be a potential nutri-genomic intervention to counter the toxico-genomic effects of the VC-CYP2E1 gene-environment interaction by inhibiting CYP2E1 activity at any given level of VC exposure and CYP2E1 status (i.e., genetically determined or personally induced) to decrease the formation of reactive intermediates responsible for the production of the DNA adducts that cause the carcinogenic mutations in VC-exposed individuals. This is consistent with preliminary experimental results. Studies of lymphoblasts from individuals of different CYP2E1 genotypes exposed in vitro to VC showed that the addition of watercress was

able to reduce the DNA damage as measured by the increased level of etheno-DNA adducts back close to baseline both in homozygous wild-type and homozygous variant individuals, although more watercress was needed to achieve this effect in the latter case as would be expected based on the epidemiologic and gene expression results noted above [Table 1]. Although much less is currently known about methods for altering DNA repair activity, similar approaches to secondary prevention based on augmenting DNA repair may be possible; for example, a recent study in mice has suggested that selenocystine administration did not protect against immediate DNA damage following ionizing radiation exposure but was nevertheless protective because it enhanced the rate of repair of the induced DNA damage. [75]

For VC-exposed individuals who have already experienced the VC-induced mutations, different molecularly targeted approaches to prevention and treatment can be employed. For example, it is now possible to restore apoptotic regulation to mutant p53. A synthetic peptide analogue for a control region of p53 has been demonstrated to cause mutant p53 to revert to normal function, reinstating its ability to cause cell death in cancerous cells and pre-cancerous cells that contain a p53 mutation both in cell culture and in animal models. [76],[77] This peptide has also been shown to effectively kill mutant p53 HAEND cells in culture that are derived from an angiosarcoma in a VC-exposed worker. Thus, this approach could potentially be used as chemotherapy for VC-exposed workers who have ASLs with p53 mutations as well as chemoprophylaxis for VC-exposed workers who do not yet have ASLs but are at high risk of developing ASLs in the future due to their increased susceptibility as evidenced by their increased rate of mutant p53 biomarkers. Similar approaches can be applied for cells with K-ras mutations. For example, the compound L-β-(5-hydroxy-2-pyridyl) alanine can cause permanent reversion of human cancer cell lines with K-ras mutations, including those that contain the VC-induced Asp 13 mutation, to a normal cellular phenotype that will no longer grow in soft agar and will no longer produce tumors in nude mice. [4],[78] Once again, such an approach could potentially be used as chemotherapy for VC-exposed workers who have ASLs with K-ras mutations as well as chemoprophylaxis for VC-exposed workers who do not yet have ASLs but are at high risk of developing ASLs in the future due to their increased susceptibility as evidenced by their increased rate of mutant ras-p21 biomarkers. The success of these approaches could be effectively monitored by following these same mutant biomarkers.

Conclusion

VC provides an instructive model for the study of carcinogenesis within the plastics industry. A detailed understanding of the molecular biology of VC carcinogenesis has provided new ways of studying the molecular epidemiology of VC carcinogenesis in exposed humans, which in turn have provided the basis for new approaches to the prevention and treatment of VC-related cancer. This model could also have much broader implications; since other potential carcinogenic exposures in the plastics' industry and elsewhere share some of the same molecular biologic pathways of metabolism and repair as VC similar molecular epidemiologic biomarkers could be useful for monitoring their carcinogenic process, and since many different types of common human cancers contain either or both p53 and K-ras mutations similar molecularly targeted approaches to chemotherapy and chemoprophylaxis could have wide applicability.

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