Relationship of Vinyl Monomers and Liver Cancers: Angiosarcoma and Hepatocellular Carcinoma*

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In the past two decades there has been a growing apprehension that new chemical compounds introduced into the environment significantly contribute to the occurrence of cancer in human beings. Concern emerging over the past quarter century that vinyl monomers carried a latent carcinogenic hazard was brought into sharp focus in 1973 by the discovery of vinyl chloride (VC) induced hepatic angiosarcoma in chemical workers.[1] Up to that point, knowledge of vinyl monomer hepatotoxicity had been derived mainly from medical case reports, from limited human experimental exposure, or from accidental occupational exposure. During the past decade, more information has been gathered from epidemic-like case occurrences, most often among industrial workers, and from extensive environmental exposure due to accidents. The realization that important, common, and frequently used synthetic materials present carcinogenic hazards has already and continues to raise very serious socioeconomic problems, not only for the workers exposed, but also the proximate public.

Physicians, especially specialists in gastroenterology and hepatology, are increasingly being asked to assess the effect of chemical exposure in both immediate and long-term situations. Historically, medicine has worked toward the diagnosis and treatment of acute, symptomatic disorders. Preventive intervention relative to asymptomatic ills caused by chronic low-grade chemical exposure is just now evolving. VC-induced hepatic injury exemplifies this process because it can ultimately lead to angiosarcoma and hepatocellular carcinoma.

BACKGROUND

Little information regarding the adverse effects of chronic vinyl monomer exposure in man was available until the 1950s, when Filatova et al[2] reported disturbances of the blood vessels and nerves in individuals continuously exposed to 20 to 300 ppm of VC. Knowledge of VC toxicity to man had been obtained previously from research workers experimenting on themselves,[3] from evaluating its suitability as an anesthetic,[4] and from studying cases of acute VC poisoning contracted during industrial use.[5] The recognition of occupational acroosteolysis in 1967 (a disorder causing bone destruction of fingertips in vinyl monomer workers) led Viola et al[6] to try to reproduce it in animals; exposure to 30 ppm of VC incidently induced cancer.[6] Maltoni and Lefemine[7] continued these studies and demonstrated hepatic angiosarcoma occurrence in rodents at 250 ppm of VC. Within months of this report, hepatic angiosarcoma was identified in two employees of a chemical plant by Creech and Johnson.[1] A review of other employees' medical histories identified four additional cases, confirming VC induction of liver tumors in man. Initial retrospective epidemiologic studies of VC workers revealed a risk ratio (risk of getting the disease if exposed versus not having been exposed) of 400:1 and a latency period of 12 to 30 years.[8] Subsequent animal studies demonstrated liver cancer induction at levels of 50 ppm. Within a year, through governmental regulation, most European and American industries reduced acceptable VC exposure levels to below 10 ppm. A steady flow of publications reporting hepatic injury and liver cancer among plastic chemical workers has led to the identification of more than 100 cases of VC-related angiosarcoma.[9,10]

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THE CHEMICALS

VC ($CH_2=CHCl$) is a halogenated aliphatic hydrocarbon structurally very similar to trichloroethylene and other inhalation anesthetics. VC is both hepatotoxic and carcinogenic. Although the majority of VC exposure comes from industrial environments, it has been used as a propellant in spray cans and identified in tobacco smoke in small amounts (16 ng per cigarette, 27 ng per cigar).[11] VC has also been observed in blood and exhaled air from patients who have received 1,3-bis-(2-chloroethyl)-1 nitrosourea.[12] Like most other chlorinated ethylene derivatives, VC forms polymers. Its polymer polyvinyl chloride was initially a suspected cause of hepatic cancer and has been reported in the postmortem liver tissue of exposed animals. However, there is no evidence of polyvinyl chloride-associated liver disease in man.[13] VC produces nearly identical acute and chronic lesions in animal models and man.[14] Therefore VC-exposed animals provide an excellent model for studying the toxic and carcinogenic mechanisms of vinyl monomers. Chronic vinyl bromide exposure in animals at levels of 250 to 1260 ppm produces identical forms of liver injury and angiosarcoma, but this has as yet not been seen in man.[14]

The important physical properties of VC include a low boiling point, high specific gravity, low solubility in water, and a half-life in air that ranges from 3 to 20 hours. Its small molecular size allows easy absorption across cell membranes even though low solubility impedes this process. More than 80% of the absorbed VC material is cleared from the blood in 10 minutes after discontinuance of exposure.

METABOLISM

The Hepatocyte

VC and related monomers enter the human body mainly via the lungs. The monomer can to a lesser degree be absorbed by the skin and through the gastrointestinal tract. Most monomers are nontoxic or mildly toxic in their native state. Almost all require metabolic activation to become cytotoxic, mutagenic, or carcinogenic. The metabolism of VC occurs mainly in the liver in a three-step process. At low concentrations (<50 ppm), VC is metabolized by the alcohol dehydrogenase system into chloroacetaldehyde and monochloroacetic acid. At levels higher than 50 ppm, oxidation by the peroxidase-catalase system appears to become operative, with chloroacetaldehyde the end product. At higher levels (>250 ppm) oxidation occurs mainly via the mixed function oxidase system (P450 monooxygenase) forming chloroethylene oxide (Chloro-oxirane).[15] This highly reactive electrophilic intermediate can spontaneously rearrange to form chloracetaldehyde, chloroethanol, or be further oxidized to monochloroacetic acid. Nonhepatocytic cells have the endoplasmic reticulum enzyme system to oxidize and detoxify VC. However, their endoplasmic reticulum system differs in its capability and may have a limited specificity for certain substrates.[16,17] These oxidative systems are saturated at acute high-exposure levels (>100 ppm) and the unmetabolized VC leaves the body via the lung unchanged.[18] The highly reactive electrophilic intermediates, chloroethylene oxide and chloroacetaldehyde, if not further oxidized are detoxified by conjugation with sulfhydryl groups or epoxide hydrase, or bound to cellular protein or nucleic acids (Fig. 1).

Detoxification by conjugation with sulfhydryl groups, mainly glutathione transferases, is an adaptive but saturable system. Electron microscopic studies in man and animals show an increase in the smooth endoplasmic reticulum occurring with increased VC oxidation and a corresponding and progressive increase in glutathione transferase activity. Both events occur without evidence of cellular toxicity.[15,19] Epoxide hydrase contributes to the degradation of these reactants, but its inhibition does not increase VC binding to either proteins or adenosine,[20] whereas glutathione inhibition by diethyl maleate (which incidently is an often used catalyst in VC production processes) increases covalent binding in both hepatocytes and sinusoidal cells.[21] Those electrophilic reactants not degraded are free to interact with regulatory proteins and impair protein synthesis, thus leading to cytotoxic injury (necrosis) or to form adducts with DNA (that is, chemical attachment to DNA). Elmore et al,[22] utilizing synthesized VC intermediates, showed that VC, chloroethanol, and chloroacetic acid are not mutagenic, whereas chloroacetaldehyde and especially chloro-oxirane, are mutagenic. Furthermore, DNA adduct formation does not occur outside the living cell. Chemical effects at the molecular level (such as inhibition of postreplication repair) can cause damage at the chromosomal level (chromosome abberations) that at the cellular level may lead to cancer, reproductive abnormalities, or death of the cell. The formation of adducts causes distortion in the symmetrical structure of the double helix of the DNA, interfering with DNA replication. Studies of the other halogenated hydrocarbons and epoxides show that the stereochemical structure of the metabolites determines binding to DNA. Specific stereochemical forms are associated with

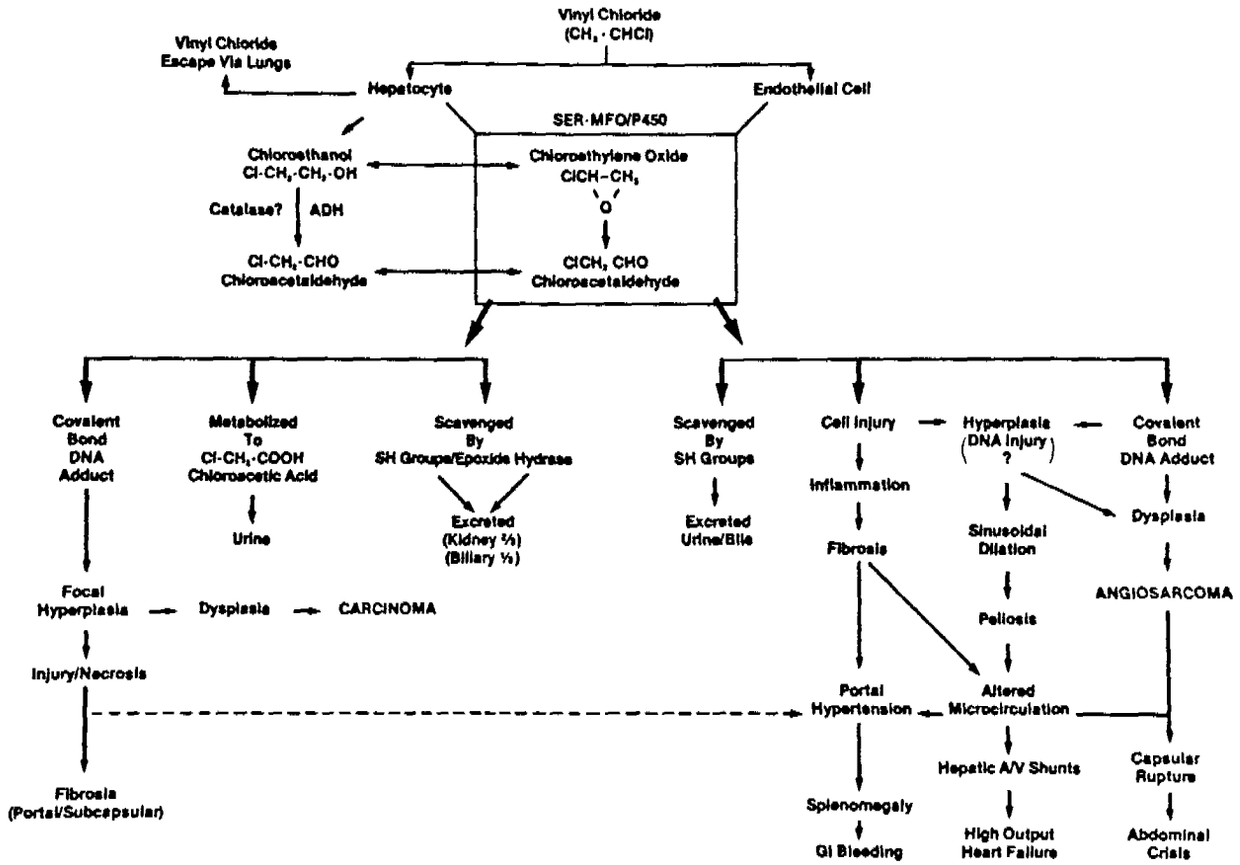

FIG. 1. Scheme outlines the metabolic fate of vinyl chloride and its metabolites at the subcellular level in the upper half and the pathologic outcome in the lower half. Left side represents the hepatocytic outcome and the right side the endothelial cell outcome. SER: smooth endoplasmic reticulum; MFO: mixed function oxidase; ADH: alcohol dehydrogenase; SH: sulfhydryl groups, such as glutathione.

impaired transcription of the genetic code and result in mutagenic or carcinogenic expressions. Chromosomal analysis of VC-exposed workers has shown higher rates of chromosomal aberrations,[23] whereas other studies[24] have not been able to demonstrate a correlation between the degree of exposure and cell chromatin aberrations. The biologic relationship of these findings to the development of the liver cancer remains to be elucidated.

The chemical carcinogenic process is also influenced by environmental or host control factors, other carcinogens, cocarcinogens, or noncarcinogenic agents (promoters). Variations in these factors often alter the ratio of activated to detoxified metabolites. An increased ratio leads to increased or synergistic effects, and a decreased ratio leads to a reduced or antagonistic action. Currently, promoters are viewed as agents that selectively or generally increase duplication of transformed cells, whereas cocarcinogens may do likewise, but they also modify the metabolism of the carcinogen. This cofactor effect is seen in VC-associated liver cancer with alcohol consumption. At low levels of VC exposure, alcohol competes with the alcohol dehydrogenase detoxifying system. This can lead to increased VC metabolism via the mixed function oxidase system and more chloro-oxirane formation. Alcohol, itself, is a mixed function oxidase enzyme inducer that can further increase formation of the VC reactive intermediates. Finally, chronic alcohol ingestion interferes with cell protein synthesis and reduces the sulfhydryl group availability for detoxification.

The Endothelial Cell

In vitro studies have shown that VC reactive intermediates are stable enough to migrate from the site of activation (microsomes) and to bind to other intracellular sites.[20] These reactants have been shown to covalently bind to both hepatocytes and sinusoidal cells.[21] Therefore hepatocytes that produce metabolites in excess may place adjacent endothelial cells, the cell of origin for angiosarcoma,[25] at higher risk of DNA injury. The nonhepatocytic cells of the liver have both the oxidative and detoxifying capability to deal directly with VC. Quantitatively, however, they have a 50 to 500-fold lower capability for detoxification of the VC reactants.[16] An additional factor placing the endothelial cell at greater risk of transformation may relate to cellular response to the carcinogen's effect on its

DNA. Hepatocytes can repair DNA lesions by endonuclease removal of the damaged region and resynthesis of the gap using the opposite strand as a template. The endothelial cell's capability for DNA repair has not yet been characterized. If the DNA repair process is operational in these cells, it may be limited in comparison to the hepatocyte.

The clinical inference with regard to genotoxic chemicals is that each step of the metabolic process (metabolism, detoxification, the stereochemical structure, and the form of covalent binding) is critical to the ultimate biologic effect. In addition, every cell type in man and each class of carcinogen have specific operational characteristics that need to be understood in order to have a rational basis for preventive action. In the near future our ability to detect clinically the formation of specific adducts and identify the site of DNA injury may allow us to influence the reparative process in order to prevent the ultimate outcome – cancer.

PATHOPHYSIOLOGY

In short-term low-level exposure the hepatocytes demonstrate no significant changes in the mitochrondrial or microsomal enzymes, protein synthesis, or glutathione utilization or content. At intermediate levels of exposure, there are no biochemical changes in transaminases, alkaline phosphatase, bilirubin, or albumin levels. However, at the subcellular level, there is a rise in glutathione reductase and a change in glucose-6-phosphate metabolism.[19,26] In long-term high-level exposure, decreased gluconeogenesis is followed by an increase in glucose-6-phosphate dehydrogenase consistent with cellular adaptation toward possible increased nucleic acid synthesis, similar to Weber and Lea's report on various carcinogens.[27]

In the initial phase of exposure there is no light microscopic evidence of hepatocellular injury; however, electron microscopy shows hypertrophy of the smooth endoplasmic reticulum, plasma membrane invaginations, and loss of microvilli (Fig. 2a). These are thought to reflect metabolite injury to the cell membranes and possibly evidence of metabolite absorption by adjacent cells, that is, sinusoidal cells. In the early phases of chronic exposure, focal hepatocellular hyperplasia is seen in the absence of single cell necrosis (Fig. 2b). Increased collagen deposition is seen first by electron microscopy in the space of Disse (Fig. 2a) and, later, histologically, at the perisinusoidal surface of the hepatocytes. At the intermediate phase of chronic exposure, associated sinusoidal cell reactions occur. The number and size of the lipocytes (fibroblast precursors) increase, and marophages are filled with phagosomes, often containing long needlelike crystals. The endothelial lining cells become larger, thicker, bulky and helmetlike in appearance (Fig. 3a). Concomitant with these endothelial and lipocyte cell changes are further increases in collagen deposition along the perisinusoidal space. Individuals with these early lesions have not shown spontaneous progression when they leave the toxic environment and/or the exposure is reduced to less than 10 ppm.[28]

Glycosaminoglycans are glycoproteins involved in collagen formation and turnover. During the intermediate to late phases of chronic exposure, glycosaminoglycans increase in both blood and urine. As the VC injury progresses, both tissue and urinary levels of glycosaminoglycans progressively increase. The various glycosaminoglycan fractions

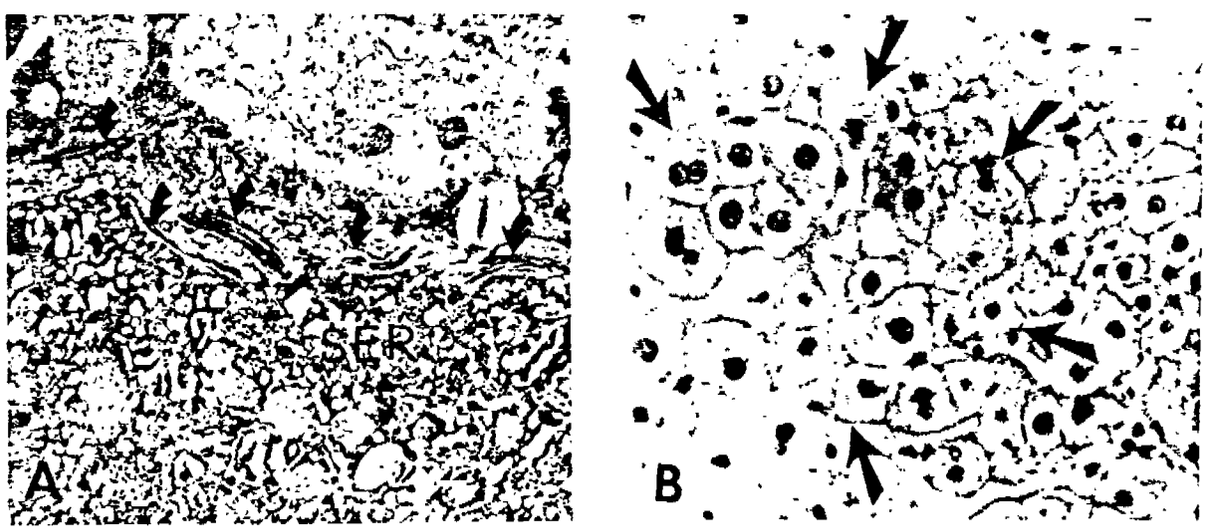

FIG. 2. A: Marked smooth endoplasmic reticulum (SER) proliferation is seen throughout the hepatocyte (lower half). Longitudinal bundles of collagen are seen along the sinusoidal surface of the hepatocytes (arrows). (Electron microscopy, × 2200.) B: Focal hepatocytic hyperplasia is seen with hepatic cell size variation (arrows). (H&E × 250.)

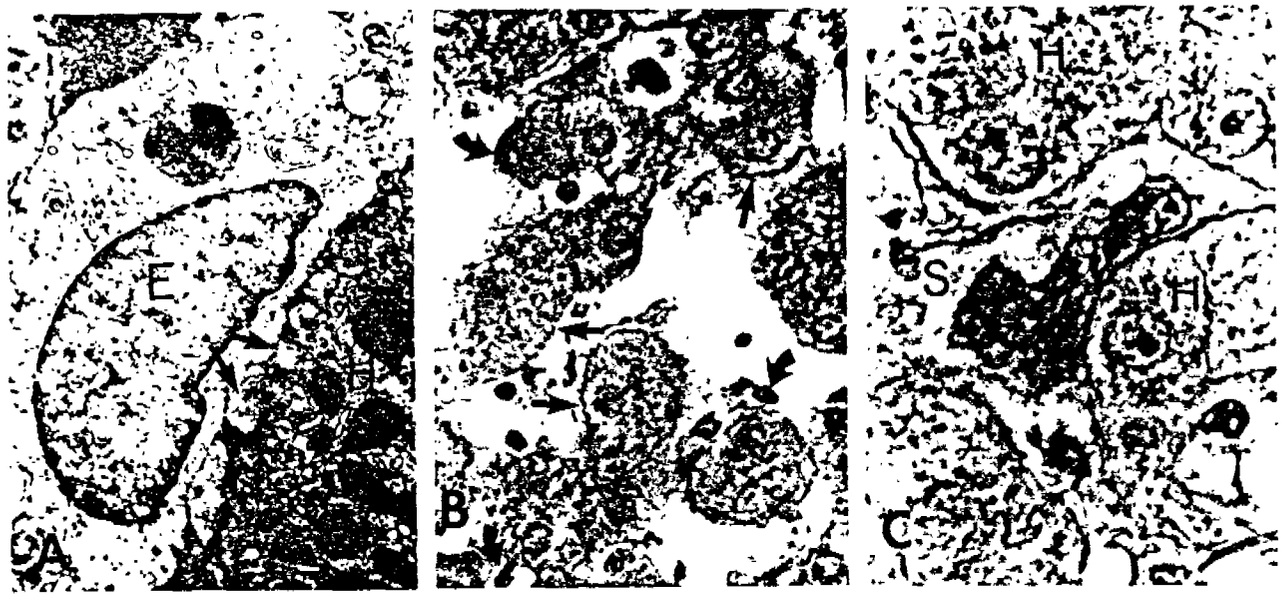

FIG. 3. A: Single, helmet-shaped endothelial cell (E) adjacent to hepatocyte (H) (right lower corner). Bundles of collagen are seen in the space of Disse between the hepatocyte surface and nucleus of the endothelial cell (arrows). (Electron microscopy, × 2500.) **B:** Dilated sinusoids with sinusoidal cell activation. Atypical cell shapes (curved arrows) are seen with increased collagen along liver cell surfaces (straight arrows). (H&E, × 400.) **C:** A single malignantly transformed endothelial cell in sinusoids (S) adjacent to normal-appearing hepatocytes (H). (H&E oil.)

shift with greater increases in hyaluronic acid and heparin than in the chondroitin sulfate fraction. These findings are similar to those found in cirrhosis and other hepatic connective tissue disorders.[29]

Sinusoidal cell activation and mixed focal hyperplasia (hyperplasia of hepatocytes and sinusoidal cells) occur in the late phase of chronic exposure. Associated with these cellular changes are focal areas of sinusoidal dilation with demonstrable increases in periportal and subcapsular fibrosis. This pathophysiologic stage appears critical in the ultimate malignant transformation of the sinusoidal cells. When these more advanced lesions are present, removal from the toxic environment does not always prevent histologic progression. In some cases the periportal and subcapsular fibrosis remains stable. Most, however, will have progression characterized by an increase in sinusoidal cell dysplasia, cellular proliferation, palisading of the endothelial cells, and worsening of the peliosis hepatis (sinusoidal dilation, Fig. 3b).

In the final phase malignant transformation to angiosarcoma is seen. Cellular transformation is multifocal in nature, that is, throughout the liver (Fig. 3c). Although the transition to angiosarcoma is associated with sinusoidal dilation and formation of large cavernous cysts, various histologic forms occur. The intralobular form involves grossly visible portions of the liver. This is associated with sinusoidal cell overgrowth and solid tumor structure. Foci of ischemic necrosis are often present due to disturbed microcirculation. The intralobular form has a less compact architecture, associated more with sinusoidal dilation; it can have aspects of papillary and/or fibrosing trabecular forms. A solid nodular form characterized by spindle or polyhedral cells is also seen and can present the greatest differential diagnostic problem, especially with a limited liver biopsy specimen.[14,30] Review of radiologic studies done 4 years before the clinical manifestation of an angiosarcoma have identified a discernable tumor and illustrated the long latency of the late phases of this pathologic process.

CLINICAL FEATURES

Chemical exposure manifests clinically in three major ways. The first and most easily recognized produces acute, clinically overt signs and symptoms. The second presentation is asymptomatic or subclinical and identified by screening or routine studies. The third and most common presentation is of nonspecific clinical symptoms and signs that, after clinical investigation, lead to the discovery of chemical exposure and its causal relationship. This last presentation is most often associated with chronic low-grade exposure.

Acute Injury

In acute VC and related chemical exposure the initial effects are manifested primarily through the system of entry: skin, lungs, and gastrointestinal tract. Clinical signs and symptoms are most often

extrahepatic, due to the physiologic effects of VC on the neurologic, respiratory, or gastrointestinal systems. Skin contact (VC in liquid form) can cause irritation and frostbite from evaporation. Neurologically, light-headedness, nausea, and dulling of visual and auditory responses can develop, resembling mild alcohol intoxication. Symptoms are directly proportional to the degree of exposure. In severely exposed individuals (8000 to 10,000 ppm) liver necrosis can also occur, but clinical and biochemical manifestations may not reach a peak for 28 to 48 hours after exposure. Clinical manifestations include disorientation, central nervous system depression, coffee ground vomitus, abdominal pain, anesthesia, somnolence, unconsciousness, and acute liver cell injury. Recovery of the less severely affected individuals is usually rapid, in terms of hours, and it is unlikely to be followed by any sequelae if recurrent exposures are prevented. In very severely exposed individuals, the extrahepatic effects can cause death (cardiac arrhythmias, respiratory arrest, among other cases).

Biochemical liver abnormalities, if present, are mild, occurring after cellular necrosis (24 to 72 hours after exposure). The most frequent abnormalities occur in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (GGTP) levels. The other liver biochemical tests (including alkaline phosphate (AP), bilirubin, albumin, and prothrombin time) are not usually affected, except in cases of severe and fulminant necrosis. After accidental or acute industrial exposures, individuals should be removed from the toxic environment and kept under biochemical surveillance for hepatic injury. Effective clinical monitoring entails following the serum transaminases (ALT, mild injury) and prothrombin time (severe injury) immediately after exposure and every 24 to 36 hours for a 72-hour period or until biochemical abnormalities return to normal. Biochemical abnormalities that persist beyond a week (without recurrent exposure) are evidence of more extensive injury requiring further diagnostic investigation.

Histologic evidence of injury (acute, mild exposures) is limited to single cell necrosis. Inflammatory cells, if present, are mainly polymorphocytic. Increased reticulum or collagen deposition in the perisinusoidal, pericentral, or periportal regions or disruption of limiting plates in the portal areas indicate recurrent or chronic exposure. The notion that a single massive exposure or a few repeated exposures can lead to cirrhosis or significant lobular distortion has neither been documented nor supported by animal studies or human observations.

Subacute Injury

Subacute injury occurs after repeated exposures to relatively smaller levels of VC (750 to 1500 ppm) and other haloethylenes. The clinical course therefore is greatly affected by the dose and frequency of exposure. Mild symptoms of fatigue, lassitude, vertigo, epigastric pain, intermittent pleuritic pain, and dyspnea have been reported. This type injury is most frequently discovered during a routine medical evaluation or during a screening program. Biochemical liver abnormalities tend to be mild, and have considerable variability. They are often ignored or attributed to recent or transient viral infection, concomitant alcohol ingestion, laboratory error, or to other medical conditions (such as obesity or diabetes).

Histologically, subacute chronic injury will demonstrate focal hepatocellular hyperplasia, often sharply outlined by increased reticulum. In later phases an increased stainable collagen (trichrome) in the perisinusoidal space can be seen (Fig. 2). The extensiveness of the sinusoidal collagen often correlates with increased portal pressure.[31]

Chronic Injury

The clinical manifestations of chronic VC liver injury are highly variable and occur after the late pathologic stages of the disease have developed.[32] The initial manifestations may vary from acute abdominal crisis to incidently discovered hepatosplenomegaly. Approximately 15 to 20% of VC-associated portal hypertension cases have esophageal varices, congested gastrointestinal mucosa, or episodes of gastrointestinal hemorrhage. Banti-like syndromes have also been found during this phase of injury.[33]

Biochemical liver abnormalities are persistent and progressive. Elevation of AP and abnormalities of bile acid (BA) levels and indocyanine green (ICG) clearances (0.5 mg/kg) are more prominent than alterations of the transaminase levels. Transaminase levels are always below 150 IU and variable, whereas AP and BA levels and ICG retention progressively increase. There is a high correlation between the specific histologic lesions of VC exposure and ICG, AP, and BA measurements.[28,34,35]

Radioisotopic scans will frequently show mild uptake abnormalities (increased uptake in the spleen or bone marrow). Up to 15% of long-term (> 7 years) exposed (> 50 ppm) workers had abnormal liver scans. Splenomegaly (> 14 cm in length), if present, is almost always associated with an abnormal liver scan.[36] In the absence of prior comparison

scans or lack of high degree of suspicion, these minor changes are often considered normal variations. Angiographic studies (arterial phase) will demonstrate puddling that persists well into the venous phase. This puddling corresponds to the sinusoidal dilation and peliosis hepatitis-like lesion found histologically (Fig. 4). Esophageal varices can frequently be identified at this stage.

Histologically, the liver will demonstrate mixed (hepatocyte and sinusoidal cell) focal hyperplasia, sinusoidal dilation with disruption of hepatic cords (Fig. 3b). The portal fibrosis present will have minimal inflammation and modest bile duct proliferation. Focal areas of subcapsular fibrosis with marked bile duct proliferation can be identified clinically on liver surface examination by laparoscopy or at laparotomy and are characteristics of chronic VC exposure.[30] Fatty infiltration occasionally seen does not appear to have any relationship to vinyl monomer exposure.

MALIGNANT TRANSFORMATION

Although all individuals with chronic injury do not develop malignancy, up to 50% have or later develop angiosarcoma of the liver. The malignant transformation is accompanied by progression of peliosis hepatis in multifocal areas, forming larger (0.5 to 2.0 cm) blood-filled, cystlike areas (Fig. 5). Extrahepatic cardiac symptoms develop due to intrahepatic arteriovenous (hepatic and portal venous) shunts causing cardiomegaly, high output failure, and widened pulse pressure. Tumor involvement of the liver is accompanied by abdominal pain, swelling, fatigue, and anorexia. The enlarged liver and spleen are tender and bruits are frequently audible over the liver.[31,32] Tumor has been found in lung, brain, bone (skull), and subcutaneous tissue. Whether these are metastases or primary sites still remains a question due to multiple organ development of angiosarcoma in animals and human histologic observations (Fig. 3c).

The tumor's solid form has a peripheral hypervascularity and a central hypovascularity, and its

FIG. 4. Hepatic arteriogram of an hepatic angiosarcoma with peliosis hepatis. Venous phase: circumferential tumor stain (curved arrows) seen about central area of hypovascularity. Scattered areas of puddling are seen throughout (straight arrows).

FIG. 5. Gross liver specimen of hepatic angiosarcoma. Sagittal cut shows multifocal blood-filled peliosis hepatis-like cysts (curved arrows). Most are peripheral. Fibrotic nodular areas are also seen (straight arrows).

peripheral tumor stain persists into the late venous phase. Puddling of dye is common and diffuse. The hepatic artery is usually not encased but may be displaced. Portal and hepatic veins are usually displaced but not obliterated (Fig. 4). On liver imaging, whether by computed tomography, ultrasonography, or radioisotopic scanning, the solid tumor usually causes a posterior and superior peripheral defect in the right lobe. Initially it can appear as a single lesion but most often is multifocal throughout both lobes of the liver. This peripheral location is consistent with the direct extension of tumor and peliosis hepatis lesions through the liver capsule. This leads to the characteristically terminal event of peritoneal hemorrhage, acute abdominal crisis, hypotension, shock, and death. Hepatic angiosarcomas can be differentiated radiologically from hepatomas, primary liver cell adenomas, cavernous hemangiomas, metastatic lesions, and macronodular regeneration due to cirrhosis or nodular hyperplasia.[36]

Hepatocellular Carcinoma and Associated Cofactors

VC and its sister monomer vinyl bromide have been mostly associated with hepatic angiosarcomas. Most xenobiotic studies in animals are associated only with an induction of hepatocellular carcinoma. There is, however, both animal[37] and human[38] evidence of hepatocellular carcinoma induction occurring in VC-exposed individuals. In at least two separate observations hepatocellular carcinoma has been found in VC-exposed workers. In these observed cases there was chronic alcohol consumption and histologic features of both alcohol and VC liver injury. In animals exposed to alcohol and VC there is a 25% increased occurrence of liver tumors of mixed histologic types, including hepatocellular carcinoma and angiosarcoma. These human cases and animal studies illustrate the change in the target cell transformation that can occur when exposure is associated with other cofactors or promoters, such as alcohol.

These cofactors may also include viruses and drugs. Of passing interest is the finding that VC-exposed workers with hepatocellular injury have a higher incidence of anti-HBs than workers without histologic evidence of liver injury. Concomitant viral infections may play a role in the induction or promotional process of chemical carcinogenesis. Recent identification of human oncogenes and the incorporation of viral DNA into human DNA give support to this concept.

TREATMENT

Surgical

Although the tumor is often multifocal, liver resection at the chronic injury or early transformation stages can be attempted if the tumor appears limited to one lobe and the degree of hepatic fibrosis is not extensive. However, in most cases resection cannot be carried out at the time of laparotomy because of the extent of the portal fibrosis and/or neoplasm.[39] One recently resected patient who is also receiving chemotherapy remains alive after 9 months.

Radiotherapy

Radiotherapy was attempted in one patient whose tumor appeared radiologically limited to one lobe and in whom resection was not considered technically feasible. No identifiable tumor response or attributable side effects were observed due to the radiotherapy. Subsequent histologic examination at time of autopsy revealed no viable hepatocytes, but many residual tumor cells in the radiated lobe. Angiosarcoma cells appear more resistant to radiation than normal hepatocytes. Radiotherapy does not appear to be a viable therapeutic alternative at this time.

Chemotherapy

VC-associated hepatic angiosarcoma has usually spread throughout the liver by the time of diagnosis. Mean duration of survival of untreated patients is 6 months. Systemic chemotherapy using multiple drug regimens has demonstrated a palliative reprieve. Five patients with histologically diagnosed angiosarcoma all received doxorubicin, 16 mg/m^2 intravenously for 3 to 4 weeks, combined with cyclophosphamide, 600 mg/m^2. Side effects of this chemotherapeutic regimen included mild thrombocytopenia and granulocytopenia with each course of treatment. One individual developed sepsis during the period of granulocytopenia. However, none of the patients died due to complications of the chemotherapy. Response to this regimen included significant changes in liver and tumor size along with an improvement in liver functional capability as measured by ICG clearance. Therapy was associated with an improved performance status lasting at least 2 months or longer. Three individuals had objective responses lasting 4, 9, and 54 months. One individual had stable disease for 10 months, and the fifth had progressive disease. Responding

patients maintained an excellent performance status during therapy. Survival from time of diagnosis ranged from 4 to 58 months, average survival being 24 months and median survival, 13 months.[40]

There is insufficient data, because of the small group of patients treated to recommend this specific drug combination for general use in hepatic angiosarcomas. Although doxorubicin appeared to be of central importance, our present experience simply indicates that chemotherapy is effective in reducing tumor size and can improve the quality and possibly the duration of survival. Further studies with combination chemotherapy are necessary.

DIFFERENTIAL DIAGNOSIS

Biochemical

Interpretation of laboratory data in screening programs, that is, in an asymptomatic working population, requires a slightly different medical orientation. Industrial populations (working populations) are as a group medically healthier than the general population. Therefore any test abnormality should be considered a significant deviation until proved otherwise. Studies of chronically exposed chemical workers demonstrated that enzyme tests (ALT, AP, AST, and GGTP) became abnormal only in the late stages of exposure, often concomitant with the clinical manifestations of the liver disease. The tests found most useful for follow-up and screening include: ALT, AST, AP, and BA levels and ICG clearance. In advanced subacute or chronic chemical injury ALT, AST, and GGTP tests provide a very high degree of sensitivity (correctly identify individuals with hepatic disease) whereas AP and BA tests provide a similar degree of specificity (correctly identify those individuals without disease).[41] GGTP, however, has a high false positive rate and should not be used alone for screening or follow-up. Low-dose ICG clearance (0.5 mg/kg) gives the single best combined sensitivity and specificity for detecting latent VC hepatic injury.[42,43] Alkaline phosphatase and fasting BA levels and low-dose ICG clearance, at present, are the most useful combination of tests for identification and follow-up of chemical liver injury in the subclinical stages.[28,33,43,44]

Radiologic Studies

Gray-scale ultrasonography was initially reported to provide more accurate detection of liver injury without radiation risks.[45] However, prospective comparison of technetium-99m sulfur colloid radioisotopic scan and gray-scale ultrasonography showed no difference in diagnostic capability with regard to histologic lesions. However, radioisotopic scans provided higher sensitivity (84%) and specificity (97%) in detecting hepatic masses. Gray-scan ultrasonography can provide a high sensitivity (75%) for identifying portal fibrosis and fatty metamorphosis through its echo patterns; however, its false positive rate (40%) and up to 30% technical inadequacy make the radioisotopic scan the more suitable method for detecting liver and spleen masses.[46] Additionally, radioisotopic scans can provide an early noninvasive method for identifying developing portal hypertension (that is, via extrahepatic uptake in the bone marrow and spleen). Computed tomography appears to provide the best overall capability of identifying hepatic masses and determining tumor density, but it cannot detect developing portal hypertension.

Environmental Triage

It is still the author's recommendation that the most cost-effective sequence for screening high-risk chemically exposed individuals for hepatic injury and potential tumor development is to begin with: ALT screening; confirm with BA and AP tests and ICG clearance studies; and if abnormalities persist, utilize radioisotopic scan and liver biopsy.[34,43,44]

Histologic Characterization of Chemical Injury

Chronic VC exposure is characterized by hepatic subcapsular, portal, and perisinusoidal fibrosis, and hyperplasia of both sinusoidal cells and hepatocytes. Initial histologic studies, mainly on autopsy material, indicated that focal hepatocellular hyperplasia (hyperplasia of the hepatocytes) might be the earliest histologic alteration indicative of exposure. This observation was substantiated in a double-blind duplicative study of liver biopsies from 34 chemically exposed workers with hepatic biochemical abnormalities. Thirty-five percent of exposed workers with biochemical abnormalities had hepatic lesions consistent with exposure, half with focal hepatocellular hyperplasia alone, the other half with focal mixed hyperplasia. In contrast, only 19% of exposed workers without biochemical abnormalities demonstrated hepatic lesions consistent with chemical exposure. None of these, however, had the more advanced lesion of focal mixed hyperplasia.[28]

Immunologic Markers

Angiosarcomatous liver tissue from VC-exposed workers displays tumor-associated antigens that are shared by normal liver and other tissues.[47] Evidence for the existence of tissue or plasma antigen induced by VC or its metabolites has also been demonstrated. However, these tissue and plasma antigens have not provided sufficient sensitivity for screening or specificity for differential diagnosis.

Lymphocyte transformation studies among VC exposed workers have shown a decreased lymphocyte response to antigens of liver angiosarcoma and normal tissue rather than the expected reactive response.[48] This lower overall lymphocyte responsiveness is supportive of the concept that chemically-induced tumors have more of an inhibitory effect on the immune function of the host than a generalized stimulation of the host's immune apparatus. The lymphocyte responses reported in nonchemical hepatocellular cancers may be more related to etiologic agents that effect the balance of the host-parasite relationship than the tumor itself.

HLA tissue typing has been evaluated for identifying individuals susceptible to chemical liver injury. Preliminary results indicate a possible increased incidence of HLA-B15 among chemical workers with liver disease, but not among the subgroup with chemical liver injury. Studies are under way to determine the value, if any, HLA-B15 may have in identifying liver injury.

CLINICAL EPIDEMIOLOGY—PREVENTION

It is generally believed that xenobiotics and other environmental carcinogens can induce hepatic cancer by a single exposure injury to DNA and that repetitive exposures to a carcinogenic agent shorten the latency time to cancer development. Both of these general principles carry great socioeconomic implications with regard to cancer risk and prevention, not only for exposed workers, but also for communities in highly industrialized areas. Support for these two concepts comes from animal studies with regard to liver angiosarcoma and hepatocellular carcinoma.[49] The 25 North American and 70 worldwide reported cases of VC-induced hepatic angiosarcoma have provided the opportunity to study this relationship. All of the VC-associated hepatic angiosarcoma cases were first exposed between 1941 and 1967.[10,50] The majority (92%) have long exposure periods (10 to 33 years); only 8% had short exposure intervals (3.5 to 7 years). The mean duration of exposure was 18.5 years, with two peaks occurring at 15 and 21 years. Latency (interval from initial exposure to diagnosis of tumor) in all of the hepatic angiosarcoma cases was greater than 9 years (range, 9 to 35 years), with two peaks occurring at 15 and 25 years after the first exposure. However, the worldwide VC-related angiosarcoma deaths, which began in 1955 and increased to more than one a year after 1967, peaked in 1976 and have subsequently been rapidly decreasing. However, because of the greater than 10-year latency period, the occurrence peak should not have been reached before 1983.

This occurrence peak first began in Canada in 1973, the United States in 1974, France in 1976, and Germany in 1978. This correlates roughly with the dates most plant operations began (in Canada in 1941, the United States in 1939-1944, France in 1941-1957, and Germany in 1952-1960). The start-up dates for plant operations are of great importance with regard to exposure levels. In the early 1940s recorded levels reached 1000 to 3000 ppm. During the 1950s, levels were reduced to 200 to 500 ppm for engineering reasons, in the early 1960s, to 50 to 500 ppm, and finally during the late 1960s and early 1970s, to 50 to 250 ppm. After 1974, federal regulations reduced the exposure levels to 1 ppm, which has been generally followed by most industrial nations.[51]

Based on the concepts that a single exposure can initiate liver cancer and that progressive exposure can shorten latency, it was anticipated that the number of VC-associated angiosarcoma cases would increase as the pool of prior exposed workers grew older. However, the opposite has occurred, and almost simultaneously with the 1974 reduction of exposure to the 1 ppm level. A further study of the relationship of exposure to latency has shown a direct correlation between total exposure and the clinical onset of hepatic angiosarcoma, but in reverse direction, that is, the higher total exposures have the shorter latencies.[52] This relationship was found in both those with long exposure periods (10 to 30 years) as well as with short exposure periods (3 to 7.5 years). The relationship is further strengthened when total exposure is weighted with regard to the years the exposures occurred (that is, exposures in the early 1950s and 1950s being weighted more heavily than those in the 1960s and 1970s). No relationship has been found, however, between angiosarcoma occurrence and VC exposure occurring after 1966 when exposure levels generally were below 200 ppm.

These epidemiologic data suggest that VC exposure, with regard to inducing liver cancer, has a biologic threshold. It appears that VC-induced angiosarcoma is unlikely to occur at levels below 50 ppm. Moderately high levels (> 200 ppm) induce

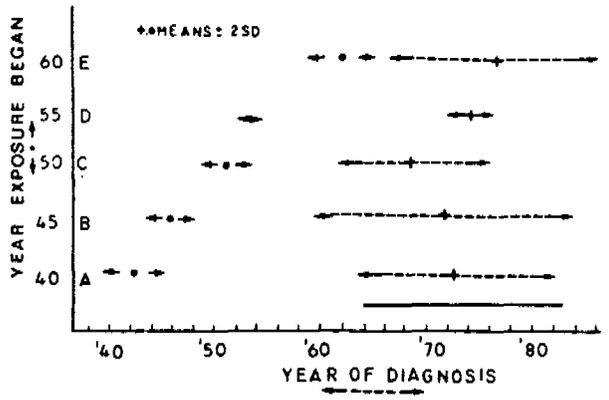

FIG. 6. Latency periods of VC—associated angiosarcoma cases in North America. Letters and solid line arrows indicate exposed groups at 5-year intervals: Groups A: 1940-1944; Group B: 1945-1949; Group C: 1950-1954, and so on. Dotted line arrows identify each group's mean and ±3 SD latency period (first exposure to diagnosis). Each group's latency period is progressively shorter, causing all the mean values to occur between 1968 and 1976, with the overall mean at 1972-1973.

hepatic angiosarcoma in the shortest time, whereas very high levels (> 1000 ppm) are mainly associated with liver cell necrosis, fibrosis, portal hypertension, and a longer cancer latency period.[53] Figure 6 illustrates these concepts and provides an explanation as to why the peak occurrence of VC-associated angiosarcoma was in 1976 and has been decreasing subsequently. The epidemiologic identification of a biologic threshold level, an optimum carcinogenic level, and a subcarcinogenic toxic level reemphasize the need to reevaluate presently held concepts regarding chemical induction of liver tumors.

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