R&S 116611

PREPRINTS

EARLY HEPATIC HISTOLOGICAL ALTERATIONS AMONG CHEMICAL (VINYL MONOMER) WORKERS

[1]Carlo H. Tamburro, [2]Laszlo Makk, and [3]Hans Popper

[1]Liver Research Center, Department of Medicine and [2]St. Anthony Hospital, [1]University of Louisville Louisville, Ky., [3]Stratton Laboratory for Study of Liver Diseases, Mt. Sinai Hospital fo Medicine, New York, New York

Portions of this work supported by NCI Contract No-1-CN-55212 and the Manufacturing Chemists Association Grant VC7.0

ABSTRACT

Earlier histological studies of industrial vinyl chloride exposure, obtained mainly on autopsy material, indicated that focal mixed (hepatocytes and sinusoidal cells) hyperplasia is the earliest histological alteration indicative of exposure. To substantiate this observation and its potential use in screening workers, 93 liver biopsies from 78 persons were investigated in double blind duplicative fashion; 35 were exposed chemical workers with hepatic screening tests abnormalities, and 13 were exposed workers without hepatic abnormalities who had liver biopsies for non-liver related reasons. A comparison group consisted of 30 individuals who were not chemical workers, but had liver biopsies for non-hepatic related reasons during the same time period. Of the exposed workers, 23 (48%) had a hepatic lesion consistent with exposure; 17 (35%) had only focal hepatocytic hyperplasia; six (13%) had focal mixed hyperplasia or more advanced lesions. In contrast, only five of the comparison group had similar findings; four (13%) had only focal hepatocytic hyperplasia and one (3%) had focal mixed hyperplasia and sinusoidal dilitation. On a subsequent biopsy, this individual was found to have angiosarcoma and a history of using hair spray containing vinyl chloride as propellant. Ten individuals had 28 multiple biopsies also read double blindly, and 10 individuals had 23 readings of the same biopsy; 21/23 (91%) duplicate readings and 27/28 (96%) multiple biopsy readings in the same individuals were identical. Only 18% of either duplicate and/or multiple biopsy readings had disagreements in their biopsy assessment. Focal hepatocytic hyperplasia, in addition to the previously described mixed hyperplasia, appears to be the earliest identifiable change consistent with chemical exposure and both lesions are present prior to the development of angiosarcoma. These lesions can be consistently identified and are useful in the screening of chemical workers for evidence of exposure.

INTRODUCTION

Industrial vinyl chloride and other vinyl monomer exposure is associated with various hepatic histological abnormalities. These include subcapsular, portal, and perisinusoidal fibrosis as well as hyperplasia of both hepatocytes and sinusoidal cells. Early histological studies, mainly on autopsy material, had shown focal mixed hyperplasia (hyperplasia of hepatocytes and sinusoidal cells) to be an early histological alteration associated with vinyl chloride exposure (1,2,3). To substantiate this observation and determine its potential use in medical surveillance screening of the exposed workers, liver biopsies from 78 individuals were studies in duplicate blind fashion to determine 1) if these early histological findings were associated with extensive vinyl chloride exposure, and 2) if these histological findings occur in non-exposed populations or were associated with other diseases.

MATERIALS AND METHODS

Liver biopsies from 48 vinyl monomer chemical workers with and without hepatic biochemical abnormalities were investigated. All 48 chemical workers had had hepatic biochemical studies performed on an annual or semi-annual basis. These included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), total bilirubin (TB), gamma glutamyl transpeptidase (GGT), prothrombin time (PT), and indocyanine green clearance (ICG). In addition, history and physical examinations, liver-spleen scans, and abdominal plain films had been performed annually. Thirteen of the chemical workers had hepatic biopsies for non-liver related reasons while 35 had biopsies performed because of screening biochemical and/or liver-spleen radioisotopic scan abnormalities.

A group of 30 individuals who were not chemical workers but had undergone abdominal surgery with liver biopsies, were used as a comparison population. All of the comparison population's liver biopsies were performed during the same three-year period, at the same hospital, by members of the same medical-surgical team, with the informed consent of the individuals. This group had similar hepatic biochemical studies with the exception of the GGT and ICG clearance. None had a history of extensive exposure of working with halogenated hydrocarbons, although one patient had a history of household contact with vinyl chloride via commercial home products.

Pathology

Earlier experiences with autopsy and animal material from vinyl chloride associated angiosarcoma have recognized certain hepatic lesions in the non-tumorous area of the liver parenchyma ( ). They include:

1. Hepatocytic changes consisting of foci of enlarged hepatocytes with increased amount of cytoplasm, and large hyperchromatic nuclei (Figure 1). These cells are intermixed with hepatocytes of normal and smaller sizes and create focal, indistinct nodular areas (Figure 2a). These areas are often associated with very slight increased sinusoidal dilitation and focal increase in the reticulin framework illustrated best by silver impregnation (Figure 2b).
2. Sinusoidal lining cell changes consisting of an increase in cell number associated with nuclear changes, particularly conspicuous in areas of sinusoidal widening. This proliferation of sinusoidal cells involves (a) Kupffer cells (macrophages) with PAS-positive granules; (b) fibroblasts with elongated, almost rectangular nuclei with loose vesicular chromatin patterns and inconspicuous

nucleoli with a moderate amount of cytoplasm which reacted on PAS staining and persisted after diastase reaction. These cells are interpreted as activated fibroblasts, are associated with an increase in fat storing sinusoidal cells and augmented perisinusoidal fibrosis tissue (Figure).

3. Fibrotic changes consisting of an excell of irregular connective tissue in the periportal zone, frequently arranged around proliferating bile ductules, and focal thickened subcapsular accummulation of corrective tissue into the parencyma (Figure 7).

These histological lesions are believed to be the three earliest hepatic findings associated with vinyl monomer chemical injury. The hepatocytic changes are referred to as focal hepatocytic hyperplasia or FHH. The sinusoidal cell changes (sinusoidal cell hyperplasia) associated with the hepatocytic changes are referred to as focal mixed hyperplasia or FMH.

For purposes of this study, early fibrotic changes were not classified since the majority of liver biopsies were of needle type, which could not be evaluated for subcapsular changes.

The liver biopsies were coded and read blindly to identify (1) histological evidence of hepatic disease; (2) any characteristics of non-chemical injury excluding steatosis; (3) steatosis with or without fibrosis and, (4) evidence of chemical injury identified by the following histological findings: a) focal

hepatocellular hyperplasia; b) focal increased reticulum associated with the hepatocytic hyperplasia; c) perisinusoidal fibrosis; d) focal hyperplasia; e) focal mixed hyperplasia associated with increased reticulum deposition; f) sinusoidal cell activation; g) sinusoidal dilitation; h) portal and capsular fibrosis; i) sinusoidal dysplasia; and, j) hepatic angiosarcoma.

The presence of focal hepatic cellular hyperplasia with focal increases in reticulum, in the absence of other evidences of hepatocellular disease or steatosis, were considered the minimum presumptive evidence of chemical exposure. The presence of focal mixed hyperplasia with increased reticulum and/or perisinusoidal fibrosis was considered evidence of chemical injury of a more advanced stage. Sinusoidal cell activation and dilitation of the sinusoids were considered evidences of chemical injury of an even further stage of advancement. The presence of subcapsular fibrosis, (on wedge biopsies only) and/or increases in both portal and sinusoidal fibrosis, with sinusoidal cell activation and sinusoidal dilitation was considered the most advanced stage of chemical injury characteristic of vinyl chloride or vinyl monomer exposure. Finally, sinusoidal cell dysplasia and/or evidence of malignant transformation, constituted definitive evidence of chronic vinyl chloride exposure and the terminal stages of injury.

Work and exposure histories were available for all the chemical workers. The work histories provide a total and average exposure data for 22 chemicals based on rank order exposure estimates. These exposure work histories consisted of an estimate of exposure to each of 22 different chemicals for all of the 350 job classifications used since the start of the chemical plant. Each of the chemicals exposure was ranked on a scale of zero to six for each of the 22 chemicals. These exposure ranking estimates were done separately

for each job for each year based on the best available information and industrial expertise. Each worker's cummulative exposure to each of the 22 chemicals was based on the various job classifications he/she held during their employment. A detailed explanation of this system and an actual in-the-field demonstration of the ability of this system to identify work-related liver injury (angiosarcoma) has been published elsewhere (4,5).

RESULTS

Thirty-seven percent (13/35) of the exposed workers with screening test abnormalities had hepatic lesions consistent with chemical exposure. Among the exposed workers without biochemical abnormalities 23% (3/13) had hepatic lesions consistent with chemical exposure. In the non-worker comparison group none of those with normal biochemical screening tests and 17/19 (89%) with abnormal biochemical screening tests had hepatic lesions consistent with chemical exposure. Of the remaining two, one had focal hepatocellular hyperplasia; the other had focal mixed hyperplasia and early peliosis hepatis. This latter individual was later found to have angiosarcoma possibly from vinyl chloride hair spray exposure. The former individual had no history of chemical exposure and/or alcohol consumption, but did have hepatobiliary tract disease in the form of biliary stones. Table 1 lists the hepatic lesions found in those with and without biochemical abnormalities for the entire group.

Reproducibility

An evaluation of the reproducibility of these biopsy readings was carried out in 17 individuals who had 32 biopsies. Seven individuals had only one biopsy (2 needle, 5 wedged) and 10 individuals had their biopsies read in duplicate. Four had needle biopsies with 9 readings and 6 had wedge biopsies with 14 readings (Table 2).

A comparison of the reproducibility of the biopsy readings is shown in Tables 3, 4 and 5. Table 3 illustrates the high degree of consistent readings between multiple biopsies in the same individual. In only one individual was one of the four biopsies read differently--the wedge was read differently from the three needle biopsies.

Table 4 makes a comparison between readings of multiple needle biopsies or multiple wedge biopsies from a single individual. In these cases there was consistent agreement in all readings.

Table 5 illustrates the consistent readings of the same biopsies over the three-year period. All the needle biopsy duplicate readings and 12 of the 14 wedge duplicate readings were the same.

At least 96% (43/45) of all readings, whether duplicate or from multiple biopsies, provided reproducible and consistent findings.

Correlation of Histological Lesions With Exposure

Biopsies from all the chemical workers were divided into three categories on the basis of final diagnosis:

1) individuals with no evidence of histological liver disease (N)
2) individuals who had liver disease but no evidence of chemical injury (LD)
3) individuals who had chemical liver injury identified by the histological criteria specified (CLI).

A comparison was made between the average vinyl chloride exposure ranking of each worker and his/her final histological diagnosis. The exposure rankings were not known to those making the final histological determination at any time during the three year period. As illustrated in Figure 6 the group of workers with evidences of chemical liver injury had the highest percentage of individuals with the highest ranking of exposure to vinyl chloride. Previous studies of

this worker cohort had shown that all the cases of angiosarcomas had an average vinyl chloride exposure ranking (AER) of 3.5 or greater. AER's of 3.5 or greater occurred in 45% of the CLI group. In contrast, those with liver disease (LD) alone, only 22% had a vinyl chloride exposure rating of 3.5 or greater and in the chemically exposed workers with no evidence of liver disease, 32% had a rating of 3.5 or greater.

Biochemical Studies

A study of the biochemical abnormalities among the two histological groups of chemical workers with evidence of liver disease is illustrated in Figure 7. The five screening tests with the best degree of sensitivity and specificity in identifying latent liver injury were reviewed. Indocyanine green clearances, at the 0.5 mg/kg dose, provides the most sensitive and the most specific of the laboratory screening study, followed by the alanine amiontransferase and aspartase aminotransferase, gamma glutamyl transpeptidase and alkaline phosphatase (6). However, specificity for chemical injury appeared to be associated more with abnormalities in the alkaline phosphatase, which was far more frequently abnormal in workers with chemical liver disease than in those with non-chemical liver injury. In contrast, the ALT, AST, GGT, and ICG were more frequently abnormal in those workers with liver disease of a non-chemical origin.

DISCUSSION

Figure 8 provides the most likely progression in the development of vinyl chloride and other vinyl monomers hepatic injury and cancer development.

These findings confirm those previously reported by Thomas and Popper (2) in their original pathological studies of vinyl chloride associated hepatic angiosarcoma and hepatic injury. Focal hepatocytic megalocytosis associated with focal increases in reticulum structure, in the absence of other evidence

of acute or chronic injury, appear to be the earliest change associated with chemical exposure. Subsequently, there occurs perisinusoidal fibrosis associated with a proliferation of fibroblasts and/or its precursor cell, the Ito cell. In later stages sinusoidal cell activation occurs involving both the macrophagic and endothelial lining cells. These changes are associated with focal sinusoidal enlargement. In the late stage mixed hyperplasia (hepatocyte and sinusoidal cells) becomes prominent, followed by sinusoidal cell dysplasia with palasading of cells, and finally malignant transformation.

Our data demonstrates that focal hepatocytic hyperplasia is the earliest consistently identifiable histological finding in industrial workers exposed to vinyl monomer chemicals. The very limited occurrence of these findings in non-chemical workers and the fact that focal mixed hyperplasia, a more advanced lesion, was only found in chemically exposed workers and in a non-chemical worker with vinyl chloride exposure (via hair spray), adds further confirmation that these lesions are related to prolonged or repeated periods of chemical exposure.

Whether these lesions should be viewed as precursors to the development of cancer or only as an early reflection of the degree and duration of chemical exposure cannot be determined at this point in time. The unchanging and stable status of screening studies over a seven year follow-up period and the histological similarities among the serial biopsies of these workers suggest that these early lesions are non-progressive when the affected individuals are removed from exposure (7).

The presence of sinusoidal cell dysplasia, however, has been associated with the later development of angiosarcoma as illustrated in one of our cases and may represent a true precancerous or a non-reversible lesion.

REFERENCES

1. Popper H, and Thomas LB. Alterations of liver and spleen among workers exposed to vinyl chloride. Annals of the New York Academy of Sciences 1975; 245:172-194.

2. Thomas LB and Popper H. Pathology of angiosarcoma of the liver among vinyl chloride-polyvinyl chloride workers. Annals of the New York Academy of Sciences 1975; 246:268-277.

3. Gedigk P, Muller R and Bechtelsheimer H. Morphology of liver damage among polyvinyl chloride production workers. A report on 51 cases. Annals of the New York Academy of Sciences 1975; 246:278-285.

4. Greenberg RA and Tamburro CH. Exposure indices for epidemiological surveillance of carcinogenic agents in an industrial chemical environment. Journal of Occupational Medicine 1981; 23:No. 5, 353-358.

5. Greenberg RA and Tamburro CH. Monitoring exposure to hazardous chemicals in an industrial setting: a method of demonstrated utility. Journal of Occupational Medicine ,198

6. Tamburro CH and Greenberg RA. Effectiveness of federally-required medical laboratory screening in the detection of chemical liver injury. Environmental Perspectives, 1981 , 41:117-122

7. Tamburro CH, Davidson CS, Fisher MM, et al. Medical surveillance for chemical hepatotoxicity, Guidelines for Detection of Hepatotoxicity Due to Drugs and Chemicals. Davidson CS, Leevy CM and Chamberlain EC (ed.) U.S. Department of HEW, NIH Pub. No. 79-313, 1979, Chapter 5; 60-80.