

Biological Effects of Vinyl Chloride: An Experimental Study

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Plasma activities of alkaline phosphatase, (AP), transaminases and total lactate dehydrogenase (LDH) with isoenzymes were determined in mice inhaling 50 and 500 ppm vinyl chloride (VC). The animals were also autopsied and the tissue pathology was studied.

The total LDH activity was elevated in both dose groups along with a shift to cathodic enzymes. AP was increased in animals exposed to 500 ppm and transaminases were not at all changed. Enzyme changes occurred after the appearance of tumors.

Alveogenic adenomas occurred in all animals at the higher dosage and in about half of the animals inhaling the lower dose. Subperitoneal and subcutaneous hemangiosarcomas were frequent in both dose groups; but especially among 50 ppm animals. Only one animal had a hemangiosarcoma of the liver. No liver fibrosis was seen. All primary subperitoneal and subcutaneous tumors were located in fat tissue. Telangiectasis was observed in two animals in the 500 ppm series. The importance of blood vessel changes in the toxicology of vinyl chloride is discussed.

Introduction

Among other toxicological effects (1), vinyl chloride (VC) has been found to induce liver injury in exposed workers (2) and has also been demonstrated to be an experimental (3, 4) and a human carcinogen (5, 6). Although a few studies on experimentally induced liver injury after chronic exposure to VC have been made, no attempt has been made to investigate both biochemical and histopathological parameters in mice. The aim of the present toxicological study was to investigate possible changes in plasma enzymes and at the same time the histopathology of laboratory mice exposed to VC.

Design of Study

Groups of mice of an outbred albino commercial strain (NMRI strain) were exposed by inhalation from 12 weeks of age to 50 and 500 ppm VC, 6 hr day, 5 days/week. Each group consisted of 12 females and 12 males.

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The 50 ppm group was exposed for 52 weeks and the 500 ppm group for 26 weeks. A control group of 24 females and 21 males was exposed to air only. The animals were observed during their life time.

The plasma activities of the following enzymes were analyzed in an LKB 8600 reaction rate analyzer at 37 °C. Alkaline phosphatase (AP) was measured on 20 μ l plasma according to Bessey et al. (7), except that 4-amino-2-methyl-1-propanol was used as buffer as recommended by Morgenstern et al. (8).

Glutamic-oxalacetic transaminase (GOT) was measured according to Karmen et al. (9), and the activity of glutamic-pyruvic transaminase (GPT) according to Wroblewski and La Due (10) on 25 μ l plasma.

Lactate dehydrogenase (LDH) was measured on 20 μ l plasma as described by Wroblewski and La Due (11), except that the pH was 7.4 in our study.

The isoenzymes of LDH were separated by disc electrophoresis and measured according to the method of Dietz and Lubrano (12). Plasma samples were diluted with a suitable volume of 40% sucrose, depending on the total LDH activity, in order to obtain an LDH activity of around 200 mU/ml in the sample. A 10 μ l portion of the diluted sample was added to the

electrophoresis tube. The stained gels were evaluated at 550 nm in a Philip Unicam spectrophotometer equipped with a gel scanner.

In all groups, four animals of each sex were taken for pathological examination after 26 weeks of exposure, and in the control group another group was examined 52 weeks after the start of the experiment. The remaining animals were autopsied when moribund or when death occurred.

Plasma Enzyme Changes

The transaminases were not significantly elevated in exposed animals at any time during the experiment. The AP activity in animals exposed to 50 ppm did not differ from normal values, but the 500 ppm group had an elevated AP activity 46 weeks after the beginning of exposure (Fig. 1).

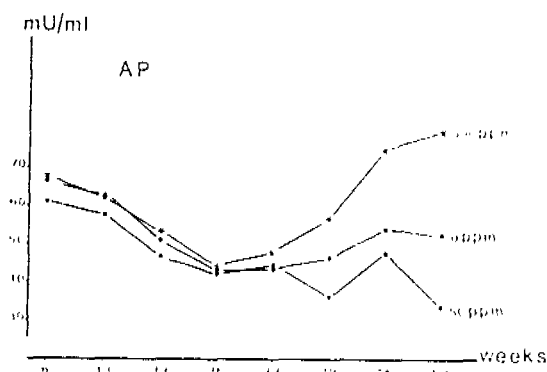


FIGURE 1. Alkaline phosphatase (AP) activity in plasma of exposed and control mice during one year. Exposure time 52 weeks (50 ppm) and 26 weeks (500 ppm). The AP activity is significantly elevated ($p < 0.05$) in the 500 ppm group at week 46. Each point represents mean values of one determination of each animal.

At 39 weeks the 500 ppm group showed significantly elevated total LDH activity, as later did the animals exposed to 50 ppm VC (Fig. 2). The increase in LDH in both groups was generally accompanied by a shift toward cathodic isoenzymes as measured by the percentage of the M form (Fig. 3).

Pathology

The details of the morphological findings in this study are to be published elsewhere (13).

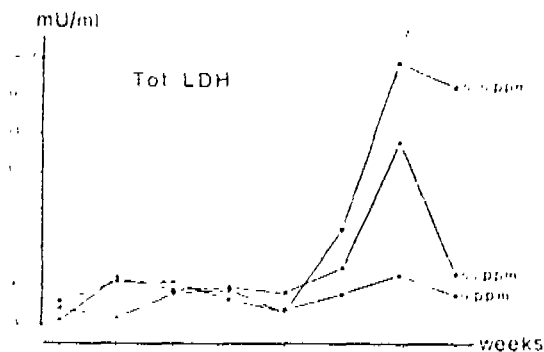


FIGURE 2. Total LDH activity in plasma of exposed and control mice during one year. Exposure time 52 weeks (50 ppm) and 26 weeks (500 ppm). The LDH activity is significantly elevated in the 500 ppm group at 39 weeks ($p = 0.001$) and in the 50 ppm group at 46 weeks ($p < 0.001$). Each point represents mean values of one determination on each animal.

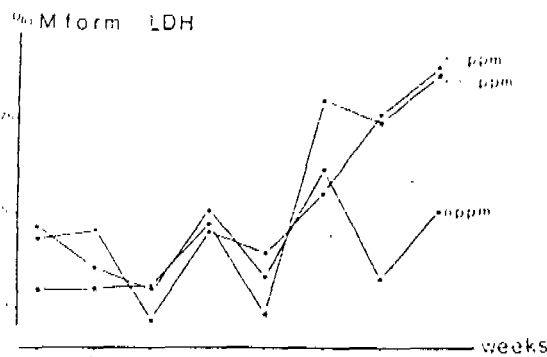


FIGURE 3. Percentage of M form of LDH isoenzyme pattern in plasma of control and exposed mice during one year. The M form is significantly elevated in the 500 ppm group from 46 weeks ($p < 0.001$) and in the 50 ppm group from 46 weeks ($p = 0.001$) compared to control.

Some morphological results are summarized in Tables 1 and 2.

In the control group three animals showed spontaneous tumors, namely, one mammary adenocarcinoma with pulmonary metastases, one disgerminoma of the ovary, and one reticulum cell sarcoma of spleen and mesenteric lymph nodes, all 45 weeks after the start of the experiment.

Table 1. Number of animals with tumors 6 months after start of exposure.

VC exposure, ppm	Number of animals with tumors			Total number of animals autopsied
	Lung adenomas	Hemangiosarcomas	Other tumors	
0	0	0	0	8
50	2	0	0	8
500	8	0	1*	8

* Mammary adenocarcinoma.

Table 2. Number of animals with tumors 12 months after start of exposure.

VC exposure, ppm	Number of animals with tumors			Total number of animals autopsied
	Lung adenomas	Hemangiosarcomas	Other tumors	
0	0	0	3*	24
50	13	14 ^b	2 ^c	24
500	24	8 ^d	5 ^e	24

* One mammary adenocarcinoma, one dysgerminoma of the ovary, and one reticulum cell sarcoma of the spleen and mesenteric lymph nodes.

^b Subperitoneal and subcutaneous and pulmonary hemangiosarcomas.

^c Mammary adenocarcinoma and one rhabdomyosarcoma.

^d Subperitoneal, subcutaneous, hepatic, and renal hemangiosarcomas.

^e Mammary adenocarcinomas and one kidney adenoma.

In the lower dose group about half the animals had alveologenic adenomas; two mice of this group were sacrificed 26 weeks after the start of exposure. In animals autopsied between weeks 29 and 56, subperitoneal hemangiosarcomas occurred in pararenal (Fig. 4 and 5) and paraintestinal sites, and in the pelvic/caudal part of the abdominal cavity as well as in brown fat or other subcutaneous tissues. The mean latency time for tumor death was 46 weeks. Ruptures of hemangiosarcomas causing hemocoelia occurred in about one third of the exposed animals in the lower dose group.

All mice in the 500 ppm group had alveologenic adenomas (Fig. 6). Also in this dose

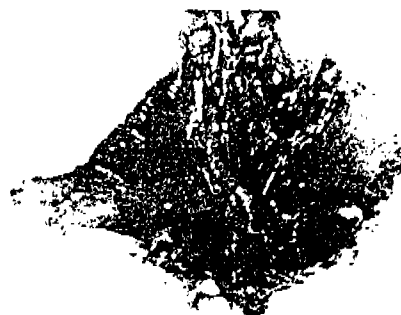


FIGURE 4. Hemangiosarcoma embedded in fat tissue is seen on each side of the uterus of animal exposed to 50 ppm VC.

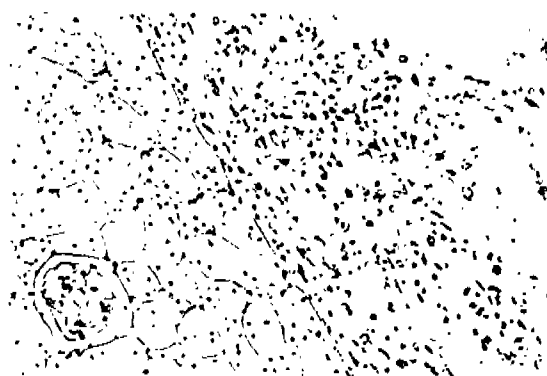


FIGURE 5. Pararenal hemangiosarcoma of an animal exposed to 50 ppm VC. The tumor tissue is well separated from kidney tissue. PAS; 171 X.

group subperitoneal hemangiosarcomas were found in the pelvic caudal part of the abdominal cavity although substantially fewer animals were bearing these tumors. Mammary adenocarcinomas were found in four animals. Only one animal had a hemangiosarcoma of the liver (Fig. 7). The mean latency time for tumor death was 35 weeks in this dose group. In two mice in the 500 ppm group blood vessel dilatation (telangiectasis) was found in the liver (Fig. 8) without any other pathological liver changes.

All primary subcutaneous and subperitoneal hemangiosarcomas were located in fat tissue in both exposed groups. No liver fibrosis was noticed in any mouse.



FIGURE 6. Alveologenic adenoma of an animal exposed to 50 ppm VC. H & E; 171 $\times$.

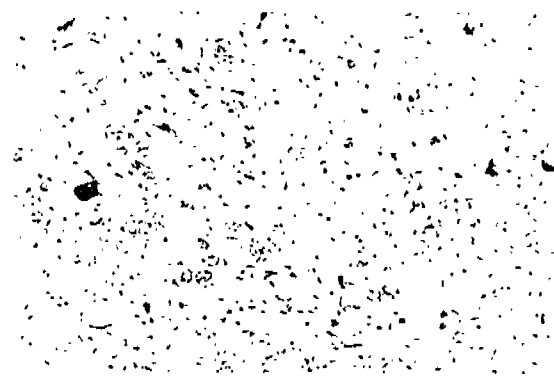


FIGURE 7. Liver of an animal exposed to 500 ppm VC showing hemangiosarcoma. H & E; 131 $\times$.

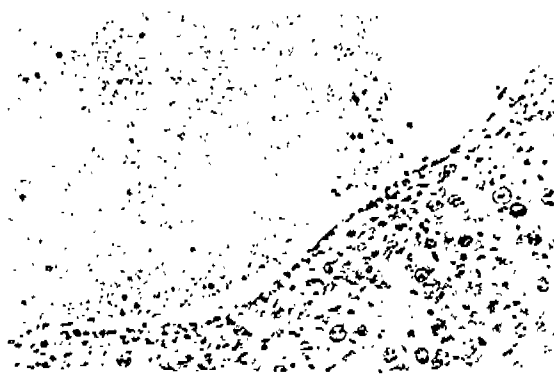


FIGURE 8. Liver telangiectasis of an animal exposed to 500 ppm VC. H & E; 171 $\times$.

Discussion

Changes in total plasma LDH enzyme activity can have several causes, such as increased physical stress (11), tissue necrosis due to disease or chemically induced organ damage (15, 16), or tumor growth (17). In connection with irreversible injury, cells are releasing intracellular material including LDH (18, 19), which may result in an elevation of total plasma LDH and/or an isoenzyme shift sometimes characterizing the organ damaged. Added to that, malignant cells leak small proteins (20) and may thus possibly contribute to a change in the total plasma LDH activity. A shift towards the M form in the LDH-isoenzyme pattern has been observed, for instance, in monkeys exposed to carbon tetrachloride (21) and in rats exposed to chlorinated pesticides (22). The isoenzyme shift in malignant exudates is usually cathodic of nature (23), but the isoenzyme distribution of plasma LDH of cancer patients seems not to be a good diagnostic tool for detecting malignancy (24).

In the present experiments total LDH was elevated in both exposed groups of animals. A tendency to a dose-effect relationship in the elevation of total LDH but not in the percentage of M form was seen. It is also interesting to note that a change in LDH activity occurred long after the first appearance of tumors, benign or malign, in both exposed groups. The increase in percentage of M form in the LDH isoenzyme pattern suggests a liver injury. There was, however, no elevation in transaminase activities, which could serve as a further indication on liver injury.

AP normally decreases with increasing age of the animal (25). An increase in AP activity indicates among other things, lesions in the hepatobiliary tract (26) and has been observed in VC-exposed workers (27). Only those animals exposed to 500 ppm showed a significant increase in AP activity.

The joint biochemical data indicate a tissue damage in mice caused by VC although the data are not convincingly indicative of liver injury. Changes in plasma enzymes in VC-exposed mice seem furthermore not to be a good diagnostic criterion of tissue injury or early malignancy, as pathological lesions are manifest long before a deviation from normality in enzyme activity is noticed (28).

Hemangiosarcomas of the abdominal sites seem often to have ruptured leading to death in hemocoelia. The blood vessels may thus have been fragilized during tumor induction or growth. In two animals inhaling 500 ppm blood sinus dilatation in the liver was seen without any other pathological liver change. In studies made on exposed workmen the blood vessels are involved as a target organ for vinyl chloride in the development of acroosteolysis and of Raynaud's phenomenon as well as it is reflected in an overrepresentation of deaths in circulatory diseases among VC/PVC workers as reported in this symposium (29). One may thus conclude that blood vessel changes are part of the so-called vinyl chloride syndrome, including tumor disease—or may even be a step in the development of malignant tumor disease induced by this chemical (30).

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