Effect of Ethanol on Vinyl Chloride Carcinogenesis

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Four treatment groups (80 male Sprague-Dawley rats/group) were used in a $2 \times 2$ factorial design: inhalation of 600 ppm vinyl chloride (VC) 4 hr/day, 5 days/week for 1 year; VC and ingestion of 5% ethanol in water (v/v); filtered air and ethanol; filtered air. Ingestion of ethanol was begun 4 weeks prior to inhalation of VC and continued for life or termination of the study at two and one-half years from the first VC exposure.

In this model system, ethanol potentiated the carcinogenic response to VC in the liver and produced an excess of neoplasms in animals receiving ethanol alone. Inhalation of VC induced angiosarcoma of the liver in 23% of the exposed animals; ethanol in addition to VC inhalation increased the incidence to 50%. Concomitant administration of VC and ethanol also produced an excess of hepatocellular carcinoma and lymphosarcoma. Ethanol with or without VC had a strong tumorigenic effect on the endocrine system. These results indicate that ethanol is a cocarcinogen in relation to the carcinogen VC.

Introduction

The relationship between inhalation of vinyl chloride (VC) and angiosarcoma of the liver is well documented in man (1, 2) and animals (3, 4). Little is known, however, of social or environmental factors which may alter the response of mammals to the chronic inhalation of VC.

Short-term studies suggest that ethanol may modify the potency of the carcinogen VC. The uptake of VC by rats in vivo was inhibited by prior administration of ethanol (5), and in hepatocyte suspensions of rat liver, 4 mM ethanol inhibited VC metabolism 50% (6).

The following study was designed to elucidate the effects of chronic ethanol ingestion as a cofactor in the carcinogenicity of inhaled VC. The pathological responses of rats to ethanol, ethanol with concomitant inhalation of VC, inhalation of VC or filtered air, were examined in this model system.

Materials and Methods

Three hundred and twenty Sprague-Dawley male rats (Harlan Industries, Indianapolis) were divided into four groups and exposed to toxic agents as indicated in Table 1. Food and water were withdrawn during VC inhalation and animals were held in compartmented stainless-steel holding-cages which were rotated in the chamber. Temperature in the 60 ft$^3$ chamber ranged from 70 to 75°F, air flow was 15 ft$^3$/min and humidity ~ 50%. The chamber effluent was mixed with natural gas and incinerated at 600-700°C; the incinerator effluent was cooled and scrubbed of HCl with water.

VC (purity 99.9%, Matheson Gas Products) was metered into filtered air prior to entering the chamber. A Baseline Industries FID 1020 BTR gas chromatograph equipped with a 10 port MPS 1610 multipoint sampler monitored VC concentrations in the chamber and effluent; analysis time, 3 min.

All rats were autopsied, either at the time of spontaneous death, when sacrificed because moribund, or at the termination of the study two and one-half years from the first exposure to VC. Tissues were fixed in neutral formalin, embedded in paraffin and cut at 6 μm thickness. Sections were

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October 1981
bodyweights of exposed depressed. After a VC-ethanol 80% 80% VC Group receiving Results contrast exposed Ethanol 80% Filtered air

were decreased by causes noma rat seen ported some Carcinoma Group 8 Ethanol VC-ethanol (VC Ethanol 600 ppm, 4 hr/day, 5 days/wk, 1 year; 5% ethanol in water (v/v) for life.

\(^a\) Numbers in parentheses denote month the first neoplasm was observed, timed from the first exposure to 600 ppm VC.

stained with hematoxylin and eosin. Special stains were used when necessary.

**Results**

Timed from the first exposure to VC, average body weights of exposed animals were slowly depressed. After 18 months the difference between exposed and control rats was about 9%. There was a more pronounced effect on the survival of exposed animals. At 18 months, survival was 40% in VC exposed and 18% in VC-ethanol exposed animals in contrast to groups inhaling filtered air in which 70% of the animals ingesting a normal diet and 73% receiving 5% ethanol survived at 18 months. The decreased survival in VC-exposed groups was due to the development of pathological lesions induced by VC or VC-ethanol rather than to nonspecific causes such as murine pneumonia.

Angiosarcomas and hepatocellular carcinomas were seen in the liver of rats inhaling VC (Table 2). In some cases, more than one angiosarcoma or carcinoma developed in an animal; the incidence reported in Table 2 represents the type of lesion per rat rather than the total number of liver lesions. The first deaths from angiosarcoma occurred in a VC-ethanol animal at 9 months and at 12 months in a rat exposed to VC alone. The incidence of angiosarcoma of the liver was increased from 23% (VC alone) to 50% in VC-ethanol exposed rats. Ethanol had a similar effect on the induction of hepatocellular carcinoma by VC; 44% developed the neoplasm from VC inhalation, and 60% developed the carcinoma from treatment with both agents. Angiosarcoma of the liver was not found in either filtered air group; however, hepatocellular carcinoma was observed in 10% of the ethanol-ingesting and in 1% of untreated animals. The incidence of "hyperplastic nodules" is included as an indicator of toxicity.

Endocrine tumors, which comprised the second largest group of neoplasms, are summarized in Table 3. Malignant tumors included seminomas in the ethanol group, a thyroid tumor in the VC-ethanol group and adrenal tumors in the ethanol and VC-ethanol groups. The incidence of pituitary adenomas in untreated animals suggests that these rats were unusually susceptible to this lesion. It appeared that VC had no influence on the development of pancreatic adenomas which consisted mostly of beta cells. The total number of endocrine tumors in each group indicates a strong effect of ethanol on the endocrine system.

The number of neoplasms occurring at other sites was small in comparison to those that developed in liver and endocrine tissues (Table 4). Lymphosarcomas were more frequent in VC-ethanol treated rats although these tumors were found in all four groups. Fibromas and fibrosarcomas occurred in the region of mammary glands, but glandular mammary tumors did not develop in the male animals. The total number of fibrous tissue neoplasms (fibromas plus fibrosarcomas in the ethanol group, 12 versus 5 and 6 in VC exposed animals) may relate to the effect of ethanol on the endocrine system.

### Table 1. Group designations and treatments.

| Group      | Number of rats | Diet                                      | Inhalation protocol |
|------------|----------------|-------------------------------------------|---------------------|
| VC         | 80             | Purina rat chow                           | 600 ppm^a           |
| VC-ethanol | 80             | +5% Ethanol in water (v/v)^b               | 600 ppm^a           |
| Ethanol    | 80c            | +5% Ethanol in water (v/v)^b               | Filtered air         |
| Filtered air | 80             | Purina rat chow                           | Filtered air         |

\(^a\) 4 hr/day, 5 days/week, 1 year.
\(^b\) Administered 4 weeks prior to VC inhalation, ad libitum, for life.
\(^c\) One animal died by accident during experiment. Effective number, 79 rats.

### Table 2. Incidence of neoplasms in the liver per rat.^a

| Group      | Carcinoma | Angiosarcoma | Carcinoma and angiosarcoma | Hyperplastic nodules |
|------------|-----------|--------------|----------------------------|---------------------|
| VC         | 35 (11.5)^b | 18 (12)      | 6 (14)                     | 25                  |
| VC-ethanol | 48 (10)   | 40 (9)       | 10 (11)                    | 22                  |
| Ethanol    | 8 (15)    | 0            | 0                          | 29                  |
| Filtered air | 1         | 0            | 0                          | 10                  |

\(^a\) Exposures: VC, 600 ppm, 4 hr/day, 5 days/wk, 1 year; 5% ethanol in water (v/v) for life.
Table 3. Incidence of endocrine tumors.

| Group         | Pituitary | Thyroid | Adrenal | Pancreas | Testes | Total |
|---------------|-----------|---------|---------|----------|--------|-------|
| VC            | 19        | 3       | 7       | 0        | 0      | 29    |
| VC-ethanol    | 12        | 1       | 5       | 12       | 0      | 30    |
| Ethanol       | 26        | 0       | 14      | 14       | 3      | 57    |
| Filtered air  | 8         | 0       | 0       | 0        | 0      | 8     |

*Exposure conditions and number of animals per group as reported in Table 1.

The number of gliomas was small but may be significant because of the rare occurrence of this type of neoplasm. Tumors at other sites were found in the kidney, epidermis, excretory pancreas and parathyroid.

The total incidence of neoplasms, malignant and nonmalignant, was highest in the VC-ethanol group expressed either as the number of animals per group with one or more tumors or as the total number of tumors per group (Table 5). In animals developing more than one tumor, frequent combinations were endocrine and hepatic or multiple hepatic tumors.

In groups exposed to VC alone, or ethanol alone, the number of animals with tumors in each group was about equal (Table 5). There were, however, two major differences in the response of rats to VC or ethanol: distribution of tumors, and the number of malignant versus benign neoplasms (Table 6). The organ distribution varied markedly: VC exposure induced 59 hepatic and 29 endocrine neoplasms; ethanol induced 8 hepatic and 57 endocrine neoplasms. Of the tumors linked to VC exposure alone, 77% were malignant. In animals ingesting ethanol, a surprising 44% of the tumors were malignant.

Table 4. Incidence of neoplasms in tissues other than liver and endocrine.

| Group         | Lymphosarcoma | Fibroma | Fibrosarcoma | Glioma | Other sites |
|---------------|---------------|---------|--------------|--------|-------------|
| VC            | 6             | 3       | 2            | 3      | 5           |
| VC-ethanol    | 11            | 1       | 5            | 0      | 6           |
| Ethanol       | 4             | 8       | 4            | 1      | 6           |
| Filtered air  | 2             | 3       | 0            | 0      | 2           |

*Exposure conditions and number of animals per group as reported in Table 1.

Table 5. Incidence of all types of neoplasms.

| Group         | Number of rats | Number of rats with tumor | Total number of tumors | % of rats with tumors |
|---------------|---------------|--------------------------|------------------------|-----------------------|
| VC            | 80            | 63                       | 107                    | 78%                   |
| VC-ethanol    | 80            | 73                       | 151                    | 91%                   |
| Ethanol       | 79            | 61                       | 91                     | 77%                   |
| Filtered air  | 80            | 16                       | 16                     | 20%                   |

*Exposure conditions as reported in Table 1.

Table 6. Summary of malignant and benign tumors resulting from VC and ethanol.

| Group         | Malignant tumors | Benign tumors | Total number of tumors |
|---------------|------------------|---------------|------------------------|
| VC            | 82               | 25            | 107                    |
| VC-ethanol    | 125              | 26            | 151                    |
| Ethanol       | 40               | 51            | 91                     |
| Filtered air  | 5                | 11            | 16                     |

*Exposure conditions and number of animals per group as reported in Table 1.
Discussion

In this animal model, ingested ethanol is a cocarcinogen in relation to VC induction of tumors in the liver. The incidence of angiosarcoma in VC-ethanol dosed rats (50%) was more than double that resulting from VC exposure alone (23%). VC-ethanol also induced a greater number of hepatocellular carcinomas (60%) than occurred in VC-treated animals (44%). The first deaths from angiosarcoma and carcinoma of the liver occurred earlier in VC-ethanol dosed animals indicating a shortened latent period.

The Sprague-Dawley rats used by Maltoni and Lefemine in 1974 (4) were more resistant to the effects of VC on the liver than the animals used in this VC-ethanol study. Following a year-long exposure to 500 ppm VC 4 hr/day, 5 days/week and observation from first VC exposure for 135 weeks, only 7 of 59 rats (12%) were reported to have angiosarcoma in the liver (4). Angiosarcomas developed in other sites such as in subcutaneous tissue, the abdominal cavity, neck, lung and uterus. Hepatocellular carcinomas were not reported. Zymbal gland carcinomas were seen in 8% of Maltoni’s VC exposed animals; none were seen in this study.

Ethanol has been reported to potentiate the toxicity of other halogenated hydrocarbons. In whole animals, a variety of alcohols increased the hepatotoxicity of carbon tetrachloride (CCl4) when ingested 16 to 18 hr prior to inhalation of CCl4 (7). Following acute exposures, CCl4 toxicity was enhanced by ethanol as judged by the elevation of serum glutamic-oxaloacetic transaminase levels. In similar studies, trichloroethylene (TCE) or 1,1,1-trichloroethane hepatotoxicity was potentiated by ethanol, but only at high concentrations of inhaled TCE or 1,1,1-trichloroethane (8).

Ethanol potentiation of VC carcinogenesis in the liver is probably due to an effect on VC metabolism. Inhibition of VC uptake (5) and metabolism (6) by ethanol suggests there is a shared step in the oxidation of the two toxic agents. The most likely candidates for competition are acetaldehyde and chloroaacetdehyde. The normal substrate, acetaldehyde, would be oxidized preferentially resulting in an increased half-life of chloroaacetdehyde.

Toxic manifestations of exposures were found which were not related to the neoplastic response. Many animals died because of neoplasms or secondary changes caused by tumors; however, death directly attributable to toxicity was not seen. The toxicity of ethanol will be discussed in another publication.

I wish to thank Frank Grande, Morris Blackstone and Josef Kurzhals for their technical assistance and Dianne Dotson for secretarial support.

REFERENCES

1. Creech, J. L., Jr., and Johnson, M. N. Angiosarcoma of liver in the manufacture of polyvinyl chloride. J. Occup. Med. 16: 150 (1974).
2. Delorme, F., and Mark, L. Angiosarcomas of the liver in workers having had prolonged contact with vinyl chloride: morphological description of the lesions. Union Med. Can. 104: 1886 (1975).
3. Viola, P. L., Bigotti, A., and Caputo, A. Oncogenic response of rat skin, lungs and bones to vinyl chloride. Cancer Res. 31: 516 (1971).
4. Maltoni, C., and Lefemine, G. Carcinogenicity bioassays of vinyl chloride. 1. Research plans and early results. Environ. Res. 7: 387 (1974).
5. Hefner, R. E., Jr., Watanabe, P. G., and Gehring, P. J. Preliminary studies of the fate of inhaled vinyl chloride monomer (VCM) in rats. Ann. N.Y. Acad. Sci. 246: 185 (1975).
6. Hultmark, D., Sundh, K., Johansson, L., and Arrhenius, E. Ethanol inhibition of vinyl chloride metabolism in isolated rat hepatocytes. Chem.-Biol. Interact. 25: 1 (1979).
7. Cornish, H., and Adefuin, J. Potentiation of carbon tetrachloride toxicity by aliphatic alcohols. Arch. Environ. Health 14: 447 (1967).
8. Cornish, H., and Adefuin, J. Ethanol potentiation of halogenated aliphatic solvent toxicity. Am. Ind. Hyg. Assoc. J. 27: 57 (1966).