

Oncogenic Response of Rat Skin, Lungs, and Bones to vinyl Chloride¹

P. L. Viola, A. Bigotti, and A. Caputo

Regina Elena Institute for Cancer Research, Rome, Italy

SUMMARY

Rats (Ar/IRE Wistar strain) exposed for 12 months to vapors of vinyl chloride developed tumors of the skin, lungs, and bones. The cutaneous tumors, which always appeared in the area in which submaxillary and parotid glands are located, have been histologically recognized as epidermoid carcinomas, papillomas, and mucocpidermoid carcinomas. The morphological characteristics of lung tumors, which occurred in a lower percentage, were mainly of the adenocarcinoma type, with the exception of a single epidermoid tumor originating from the epithelial covering cells. In a minor number of rats, a large proliferation of cartilaginous tissue diagnosed as osteochondroma developed in the metacarpal and metatarsal regions of the four limbs.

INTRODUCTION

The oncogenic properties of some chemical organic compounds used for the preparation of "plastics" have been widely investigated, and their limits and effectiveness have been well established. Detailed information on this subject may be found in the literature (5, 6, 8); however, all information and references are exclusively related to highly polymerized compounds of roughly the same size as used in various industries.

Oncogenic polymers produce sarcomatous tumors, with the exception of polyurethans which, as reported by Hueper (6), also induce adenocarcinomas. Recently, it has been demonstrated that sarcomatous tumors (1-4, 7) are transplantable, and it has been suggested that they may originate from the tissues of the capsule that gradually covers the plastic film. Thus, *Brondi et al.* (2-4) have observed premalignant areas made of poorly differentiated fibroblasts which were firmly attached to the plastic film up to the moment of malignant transformation.

This investigation demonstrates that even the monomer vinyl chloride possesses oncogenic properties when used with an appropriate model different from the models previously reported by several authors (9-12). The carcinogenic response

to vinyl chloride follows a singular pattern for the different tissues and organs of the rat.

MATERIALS AND METHODS

The experiments were performed with vinyl chloride ($\text{CH}_2=\text{CHCl}$, the monohalogenate derivative of ethylene) of commercial grade (99% purity) assumed to contain insignificant amounts of various noncarcinogenic contaminants.

Three-month-old Wistar (Ar/IRE) male albino rats (about 150 g body weight) were exposed to vinyl chloride vapors for 4 hr a day, 5 days a week, for 12 months. The animals were kept in metal or plastic, air-tight cages in which a constant flow of air, containing 3% v/v (equal to 30,000 ppm) of vinyl chloride, was introduced. Twenty-five rats of the same strain were the control group. At the end of the treatment, the surviving animals were killed at 20-day intervals, and the most important tissues and organs were examined histologically by standard methods. During the period of exposure, the animals were slightly soporific; however, the first few months of treatment were well tolerated and no changes in growth or behavior were noticed. After 10 months of treatment, some animals began to show a hard mass in the parauricular region which became progressively larger until it reached the size of a walnut or slightly larger. In most cases, the swelling was unilateral; it was bilateral in only a few animals. After 1 or 2 months, the growing masses became ulcerated and discharged necrotic debris, while a certain amount of tumorous tissue began to form on their surfaces. In addition, we observed that the masses were an integral part of the parauricular region and could not be distinguished from the local tissues. Caseous necrotic zones were found. Pleura and pericardium often showed diffuse inflammation of a fibrous nature and, in many cases, the lungs were covered with a number of white formations as large as grains of rice or even larger and harder than the lungs themselves. In 2 cases, the lungs were hemorrhagic with milky, thick fluid in the pleural cavities. The liver was sometimes increased in size and very fragile. The animals were subjected to X-ray analyses at different intervals from the beginning of treatment in order to control the state of the skeletal bones.

All the animals that inhaled vinyl chloride showed a series of parenchymal lesions. Among these, most prominent were the disappearance of granular and Purkinje cells, degeneration

¹A preliminary report of the results reported here was given at the Tenth International Cancer Congress, Houston, Texas, May 22-24, 1970.

Received July 31, 1970; accepted January 12, 1971.

e cerebellum, severe chronic hepatitis, interstitial pneumonia, and moderate swelling of the kidney parenchyma, assuming the pattern of tubulonephrosis.

rates of survival and the main findings are summarized in Table 1. Almost all the animals developed tumors of the lungs. Very few animals developed bone tumors; in most cases, the tumors were localized in the metacarpal and tarsal bones of all 4 extremities. Skin tumors were by far the most frequent, amounting to 65 or 70%.

Tumors. The tumor that developed most frequently in the subauricular region was the epidermoid carcinoma, but we also observed papillomas and rarely mucoepidermoid carcinomas. In all cases, the neoplasms were of epithelial origin.

The 3 patterns noticed cannot be compared to various types but rather to a transition of one type into the other, which is more likely, to different stages of the same histological type.

Warty subauricular growths occurred in some rats. The histological picture showed papillar epithelial proliferation, with progressive increase in the thickness of the epidermis (Fig. 1).

The papillary warts of exophytic type had a fibrous vascular stroma and various degrees of inflammatory lymphocytic infiltration, marked hyperkeratosis, parakeratosis, acanthosis, and areas of individual dyskeratosis or pearl-like horny formations.

The epithelial cells of the penetrating columns (Fig. 2) were irregularly arranged and were frequently accompanied by an inflammatory infiltration of the dermis. The typical features of the Malpighian layer and of the stratum corneum of the prickly cells and of germinal layers were easily recognized (Fig. 3). The horny layer was composed largely of cell nests, which appeared at certain points within the epithelial masses and assumed the well-known appearance of "horny pearls" (Fig. 3, arrows); they were made of flattened and compressed prickly cells without nuclei and were located around a central core of keratin.

Table 1

Oncogenic effects of inhaled vinyl chloride as a function of time

The animals were exposed to vinyl chloride vapors for 4 hr a day, 5 days a week, for a total of 12 months, in a constant flow of air containing 3% v/v vinyl chloride.

Rat	Survival rate	Tumors		
		Skin	Lungs	Bones
1	300	Mucoepidermoid carcinoma	Adenocarcinoma	Osteochondroma
2, 3	280-300	Epidermoid carcinoma, keratinizing type	No tumor	No tumor
4	310	Epidermoid carcinoma, keratinizing type	Adenocarcinoma	No tumor
5	333	Papilloma, keratotic type	No tumor	Osteochondroma
6	337	Epidermoid carcinoma	No tumor	Osteochondroma
7	347	Epidermoid carcinoma	No tumor	No tumor
8	347	Mucoepidermoid carcinoma	No tumor	Osteochondroma
14	354	Epidermoid carcinoma	No tumor	No tumor
16, 17	359	Epidermoid carcinoma	Adenocarcinoma	No tumor
21	350	Epidermoid carcinoma	No tumor	No tumor
22	380	Epidermoid carcinoma	Adenocarcinoma	Osteochondroma
23	380	Epidermoid carcinoma	No tumor	No tumor
24	380	Epidermoid carcinoma	Mucus-producing adenocarcinoma (alveolar cell carcinoma?)	No tumor
25	380	Epidermoid carcinoma	No tumor	No tumor
26	380	Epidermoid carcinoma	Squamous cell carcinoma	No tumor

Keratinization was irregular and often parakeratosis was observed in the areas of the tumor in which the cells were loosely aggregated and undergoing an individual rather than a collective type of keratinization (Fig. 4). In dyskeratotic areas, a few epithelial cells tended to form pearls. The tumor seldom showed an undifferentiated growth with cellular pleomorphism and several mitotic figures (Fig. 5). A few tumors showed little nests of isolated pale cells (Figs. 6 and 7) of 3 types: mucin-producing cells (originating from the duct epithelium of sweat glands or salivary glands); squamous cells; and intermediate cells with minor tendencies towards differentiation.

Respiratory Tract. These tumors, although occurring rarely, were mainly adenocarcinomatous. Only in 1 rat did we observe an epidermoid tumor originating from the epithelium-covering cells. Sometimes, the tumors were seen in their early development and consisted predominantly of cubic or columnar cells arranged as regular or irregular tubular and papillary elements (Fig. 8) and supported by poorly developed fibrous stroma (Fig. 9).

In other cases, glandular structures were often imperfectly formed and appeared as sheet-like proliferations of undifferentiated or pleomorphic character. The cells showed a tendency to extend into the pulmonary parenchyma, thus simulating the microscopic features of the so-called alveolar cell carcinoma (Fig. 10). Sometimes, this was the prevailing pattern. The pulmonary air sacs were limited by 1 or more layers of cubic, columnar, or polyhedral cells with abundant cytoplasm that was faintly eosinophilic (Fig. 11), frequently, adenopapillary excrescences spreading into the alveolar spaces were present.

In some areas, foci of cellular polymorphism with hyperchromatic nuclei were noticed; mucin was produced in varying amounts and secreted into the lumen of the tubules and acini. There were small pools of mucin in which signet ring cells occurred, either alone or in small clusters (Fig. 12). The alveolar walls were often quite thick and, at times, showed an inflammatory infiltration.

As mentioned before, a single tumor showed squamous structures and appeared to have been formed mainly by spindle and oval undifferentiated cells (Fig. 12).

Bones. In the metacarpal and metatarsal regions of the 4 limbs, a large proliferation of cartilaginous tissues arose outward from the periosteum and, from the appearance of its cells, seemed to derive directly from the cortical bone (Fig. 14). The periosteum also grew and in some areas spread as finger-like prolongations into the newly formed cartilage (Fig. 15). In these places, a gradual transition between fibrous cartilage, periosteum, and bone could be noticed.

The newly formed cartilage appeared irregular with atypical areas; the cells, which had nuclei larger than the normal chondrocytes, lay in well-formed, capsulated lacunae. The cells occurred singly, in pairs, or in tetrads and, although of different size and shape, they usually contained a single, darkly stained nucleus. The cartilaginous growth was not homogeneous, as shown by the extension of the finger-like prolongations into the boundary of newly formed tissue. Besides the chondroblastic, chondrocytic, and angiomatous areas which indicated a rapid growth, there were also

cartilaginous zones possessing regressive features, such as fibrosis and hyalinosis. The perichondrium appeared often as a compressed and structurally altered fibrous tissue.

In some cases, the tumor growth was related to the stage of the endochondral ossification occurring below the "epiphyseal plate." Ossification was irregular, so that osseous trabeculae varied greatly in thickness and contours. In other cases, foci of active cellular proliferation, cartilaginous areas, chondroid developmental nests, and calcified bones occurred in the deeper portions of the trabeculae (Fig. 18).

The new formation appeared to be an osteochondroma and consisted essentially of a bony protuberance capped by cartilage and a fibrous layer, which represented the perichondrium.

The fibrous layer was continuous with the periosteum of the adjacent cortical bone and extended inward to form septa separating and enclosing lobules of cartilage.

Control Animals. The control rats were kept under the same conditions as the experimental rats and at the appropriate time were subjected to a constant flow of air without vinyl chloride. None of these animals developed tumors or the types of parenchymal lesions developed by the rats that inhaled vinyl chloride. In a very few of the control rats, there was a swelling of the liver and kidneys.

DISCUSSION

As in many carcinogenesis studies in which multiple tumors arise in different tissues and organs, the data must be evaluated in terms of a more prompt positive response to be obtained with the ideal concentration of the carcinogenic compound. The cutaneous system is the most susceptible to the oncogenic effects of vinyl chloride.

Our experimental data do not explain why the cutaneous tumors developed in the same site, i.e., the region including the area in which the submaxillary and parotid glands are located. It is possible that the salivary glands may be involved in the concentration or excretion of vinyl chloride or some of its active decomposition products. This hypothesis is strongly supported by morphological findings showing the specific tendency of the developed tumor to appear as a mucoepidermoid carcinoma, which indicates the active contribution of the mucus glandular cells to the tumorous growth. Such kinds of histotypes are often related to some glandular aggregates, and, therefore, the histogenesis of human mucoepidermoid carcinoma has been restricted to the cells of intercalated ducts. This hypothetical interpretation must be confirmed by future experiments in which the action of the vinyl chloride should be restricted to the salivary system. At the concentrations used, saturation and wetting of the fur might well be expected. Under these circumstances, the natural cleansing habit of the rat might add a significant ingestion problem with subsequent concentration of vinyl chloride in the salivary glands. The local extreme concentration could be the result of the difficulty of a complete cleansing by the rat.

The neoplastic response of the lower respiratory tract, although of lesser magnitude, is of relevant interest, since some

cells were morphologically similar to those described in the literature. The hypothesis that mucus-producing cells may be of retaining vinyl chloride or its decomposition products to be relevant once more. Obviously, a correlation is required for the pathogenesis of the tumors and for their simultaneous incidence at the level of the lungs.

REFERENCES

1. H. and Bates, R. Prevalence of Heteroploidy in Plastic-Induced Primary Sarcomas. *Brit. J. Cancer*, 20: 555-563, 1968.
2. G. and Bueen, L. Polymer Tumorigenesis: Multiple Clonal Populations in Priority Order with Clonal Inhibition. *Proc. Exp. Biol. Med.*, 128: 1153-1157, 1968.
3. G. Bueen, L., and Brand, I. Malignant Transformation and Mutation in Non-dividing Cells during Polymer Tumorigenesis. *Proc. Exp. Biol. Med.*, 124: 675-678, 1967.
4. G. Bueen, L., and Brand, I. Carcinogenesis from Polymer Plastic: New Aspects from Chromosomal and Transplantation Studies during Premalignancy. *J. Natl. Cancer Inst.*, 39: 663-679, 1967.
5. Druckrey, H. Experimentelle Beiträge zum Mechanismus der Carcinogenen Wirkung. *Arzneimittel-Forsch.*, 1: 383-393, 1951.
6. Hueper, W. C. Cancer Induction by Polyurethan and Polysilicone Plastics. *J. Natl. Cancer Inst.*, 33: 1005-1027, 1964.
7. Johnson, K., Bueen, L., Brand, I., and Brand, G. Polymer Tumorigenesis: Clonal Determination of Histopathological Characteristics during Early Preneoplasia; Relationships to Karyotype, Mouse Strain, and Sex. *J. Natl. Cancer Inst.*, 44: 785-793, 1970.
8. Kogan, A. Experimental Blastomogenesis Caused by Synthetic Polymers (Plastics). *Meditsina, Moscow*, 1: 215-237, 1965.
9. Mastromatteo, E., Fisher, A. M., Christi, H., and Danziger, D. Acute Inhalation Toxicity of Vinyl Chloride to Laboratory Animals. *Am. Ind. Hyg. Assoc. J.*, 21: 394-397, 1960.
10. Torkelson, T. R., Oyen, F., and Rowe, V. K. The Toxicity of Vinyl Chloride as Determined by Repeated Exposure of Laboratory Animals. *Ind. Hyg. J.*, 354-361, October 1961.
11. Van Duren, B. L., Sivak, A., Gohlschmidt, B. M., Katz, C., and Melchionne, S. Carcinogenicity of Halo-Ethers. *J. Natl. Cancer Inst.*, 43: 481-486, 1969.
12. Viola, P. L. Pathology of Vinyl Chloride. 16th International Congress of Occupational Health. (Communication No. 38), Tokyo, 1969.

Photomicrographs of the original article are not being distributed with this copy because of the poor quality of their reproduction.

1. 1 to 12. All sections were stained with H & E.
1. Squamous cell papilloma of the skin. X 25.
2. Transitional stage from papilloma to infiltrative type of cancer. X 80.
3. Finger-like trabeculae branch to form secondary processes showing horny pearl formation (arrows). X 25.
4. Dysplastic area. Few epithelial cells with a tendency to form whorls. Little capacity to develop into horny pearls. X 250.
5. Atypical cells partly lacking prickles and showing considerable variation in size, shape and many mitotic figures. X 250.
6. Macropidermoid skin tumor. Occurrence of hydropic epidermoid cells, basal cells, columnar cells and oxyphilic cells in various zones. X 100.
7. Macropidermoid skin tumor, showing differentiation of masses of squamous epithelium from columnar cells, lining and proliferating interstitial spaces. X 100.
8. Micronodular adenocarcinoma of the lung arising from a segmental bronchus. X 25.
9. Detail of Fig. 8 showing tubular aggregates made up by cells with hyperchromatic nuclei.
10. Nodular bronchiolar alveolar mucus-secreting adenocarcinoma. X 100.
11. Detail of Fig. 10, showing an alveologenic pattern. X 250.
12. Mucus-producing cells with hyperchromatic and pleomorphic nuclei and occurrence of signet ring cells. X 250.
13. Squamous cell carcinoma of the lung. X 100.
14. Osteochondroma developing as a protuberance from the small bones of metacarpus and metatarsus. X 25.
15. Magnification of Fig. 14. The protuberance consists of bone trabeculae, capped by cartilage, and a fibrous layer functioning as endosteum. X 100.
16. Osteochondroma. Cartilaginous growth characterized by finger-like proliferation. X 100.
17. Osteochondroma. Area of endochondral ossification. X 100.
18. Transitional area of an osteochondroma showing the transition from cartilage to osteoid. X 100.