INDUCTION OF LUNG TUMORS IN MICE WITH DIAMYLNITROSAMINE

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UDC 616-006-092.9-02:615.277.4

Diamylnitrosamine was injected subcutaneously into CC57W mice once a week, in a dose of 0.025 ml per mouse, 17 times altogether. The animals were killed 27 weeks after the experiment began. Lung tumors (mainly adenomas, but in three cases adenocarcinomas) were found in 14 of the 17 mice which died and in all 24 of the killed animals. One case each of marked proliferation of the interstitial cells of the kidney of a precancerous character, nephroblastoma, carcinoma in situ, and invasive carcinoma of the urinary bladder also was found. Administration of diamylnitrosamine can be recommended as a method of inducing experimental lung tumors in mice.

KEY WORDS: diamylnitrosamine; tumors of the lungs.

Various aspects of the carcinogenic action of N-nitroso compounds, many of which are powerful carcinogens and induce tumors selectively in various organs of animals, an important factor when experimental models of chemical carcinogenesis are desired, have been extensively investigated in recent years. In this connection we were interested in studying the carcinogenic action of diamylnitrosamine (DANA) which, as was shown previously [1], induces lung tumors in rats when injected subcutaneously. It must be emphasized that the development of different models of pulmonary carcinogenesis is without question of both practical and theoretical importance in connection with the high frequency of tumors in this situation in man. For a more complete analysis of the carcinogenic action of DANA, it is necessary to investigate its effect on other species of animals and, in particular, on mice which are frequently used in the experimental study of pulmonary carcinogenesis.

The object of this investigation was to study the carcinogenic action of DANA on mice and to ascertain the prospects for obtaining a new model of pulmonary carcinogenesis.

EXPERIMENTAL METHOD

Experiments were carried out on 82 male CC57W mice weighing 18-20 g. DANA* was synthesized at the Laboratory of Organic Synthesis of the Professor N. N. Petrov Research Institute of Oncology [2]. The carcinogen was injected subcutaneously into the mice once a week in a dose of 0.025 ml per mouse 17 times: from the 1st through the 4th and from the 11th through the 23rd week of the experiment. From the 5th through the 10th week of the experiment the injections were interrupted because of death of some of the animals from the toxic action of the compound. The mice were killed 27 weeks after the beginning of the experiments. Organs and tissues of the dying animals were examined macroscopically and microscopically.

EXPERIMENTAL RESULTS

In all the mice in the early stages of the experiments and in some of the animals killed at its end changes connected with the toxic action of DANA were found. The surface of the liver was noticeably yellow in color,

*The authors are grateful to Assistant Scientist B. O. Kraiz for synthesizing the DANA.

Laboratory of Chemical Carcinogenic Agents, Professor N. N. Petrov Scientific-Research Institute, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Serebrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 1, pp. 71-73, January, 1977. Original article submitted June 8, 1976.

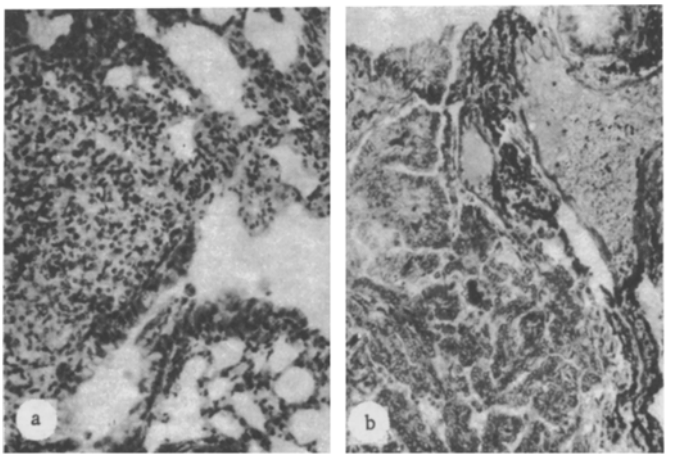

Fig. 1. Tumors of the lungs induced in mice by DANA: a) trabecular adenoma; on the right of the adenoma is a focus of proliferation of the alveolar epithelium; b) papillary adenocarcinoma. Hematoxylin–eosin, 230×.

the kidneys were somewhat enlarged and their surface was nodular and pale pink or sandy yellow in color, and in some cases numerous thin-walled vesicles formed on them, and when opened they were seen to be cavities up to 3 mm in diameter.

The first neoplasm (a tumor of the lung) was found in a mouse which died in the 17th week of the experiment. Altogether 41 mice survived until this time. Later, neoplasms of the lungs were found in 14 of the 17 mice which died before the end of the experiment and in all 24 animals that were killed. The neoplasms had the appearance of pale gray nodules up to 3 mm in diameter. The number of tumors in the mice which survived to the end of the experiment varied from 4 to 36 per animal and the mean number of neoplasms per mouse was 14.6. Multiple metastases of a tumor of the lungs in the parietal pleura and mediastinal lymph nodes were found in one mouse. Slight enlargement of the spleen was noted in 18 mice investigated at the end of the experiment. In nine animals the walls of the urinary bladder were thickened. In one mouse a tumor measuring $5 \times 3 \times 3$ mm was found in the lumen of the urinary bladder, filling it completely and invading its wall.

On microscopic examination foci of degeneration and necrosis of the hepatocytes were found in the liver.

Examination of the kidneys revealed degeneration and necrosis of the tubular epithelium, cystic dilatation of the tubules, with many albuminous casts in their lumen, and foci of proliferation of interstitial cells in the cortical layer. Where there was proliferation of the interstitial cells, foci of proliferation of the tubular epithelium consisting of cells with pale cytoplasm also were observed. In two mice there was marked proliferation of interstitial cells with the formation of bands of fibroblasts, surrounding the tubules and causing death of the epithelium. Proliferation of the epithelium with signs of polymorphism and atypia of the cells were seen in the residual tubules, and some cells could be seen with hyperchromic nuclei and condensed cytoplasm. Proliferation of the epithelium led to the formation of papillary outgrowths facing the lumen of the tubules. Foci of invasive growth of fibroblasts were found in one mouse, indicating the formation of a nephroblastoma (adenosarcoma).

In the lungs of all the mice, starting from the third month of the experiment, multiple foci of proliferation of the alveolar epithelium were observed. Against this background, starting from the fourth month of the experiment, adenomas began to appear, mainly trabecular (Fig. 1a), and less frequently tubular and papillary in structure. A characteristic feature of the adenomas was the absence of any clear boundaries or of a tendency to form a capsule. In three mice adenocarcinomas of the lung were found with marked polymorphism of the cells, and with invasion of the surrounding tissue, the bronchi, and the blood vessels (Fig. 1b).

In the urinary bladder proliferation of the epithelium was observed, with the formation of carcinoma in situ in one case and of invasive carcinoma in another. The tumor appeared against the background of a focus of hyperplasia of the transitional epithelium and it consisted of pale round or fusiform cells with large nuclei. The neoplasm had a solid structure.

In addition, diffuse hyperplasia of the lymphoid tissue with obliteration of the normal pattern was observed in the spleen of the mice. Lymphoid infiltration also was found in the kidneys and, less frequently, in the liver and lungs, evidence of the formation of lymphatic leukemia. Similar changes also were observed in some of the intact animals.

Injection of DANA into mice, just as into rats, thus led to the development of multiple tumors in the lungs. The neoplasms developed in all animals after a relatively short time; the first adenomas were formed as early as 3-4 months after the beginning of the experiments. Subcutaneous injection of DANA can accordingly be recommended for the induction of experimental lung tumors in mice.

It must also be pointed out that some of the animals developed proliferative changes in the kidneys and urinary bladder (carcinoma of the bladder in two cases and a malignant tumor of the kidney in one case). Possibly by reducing the dose of carcinogen the toxic effect would be diminished and a more selective action of DANA on the lungs would be obtained.

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CHANGES IN THE IMMUNOLOGIC STATUS REFLECTED IN THE PHYSICOCHEMICAL CHARACTERISTICS OF CELLS OF THE IMMUNOCOMPETENT SYSTEM DURING CARCINOGENESIS

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UDC 616-006.6-02:615.277.4]-07:[[616.428+616.438]-097.3-07

The state of the cell population of the lymph nodes and thymus was studied by the fluorescent probe method in rats with sarcoma of the thigh induced by dimethylbenzanthracene. Statistically significant changes were found in the uptake of the negatively charged probe 1-aniline-8-naphthalene-sulfonate (ANS) in cell suspensions depending on the stage of development of the sarcoma. In the early stages of carcinogenesis cell forms with a less hydrophobic surface and containing fewer binding sites were observed to appear. It is suggested that these cells belong to the immature category. It is concluded that the fluorescent probe method is suitable for recording changes in the immunologic status of the organism during carcinogenesis.

KEY WORDS: chemical carcinogenesis; lymphocytes; fluorescent probes.

Changes in the functional behavior of lymphocytes [6], in the morphological composition of the lymph nodes and thymus [4, 5], and also in the fine surface structure of these cells during carcinogenesis [3] has been studied and described. On the other hand, it is stated in the literature that cells at different stages of differentiation or function are characterized by a definite density of their surface negative charges [8], de-

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 1, pp. 73-75, January, 1977. Original article submitted May 21, 1976.