DOSE-DEPENDENT EFFECT OF BENZ(a)PYRENE ON EMBRYONIC LUNG ORGAN CULTURES FROM MICE RESISTANT AND PREDISPOSED TO PULMONARY NEOPLASIA

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UDC 616.24-006.6-02:615.277.4:665.44].015.3]-07

KEY WORDS: benz(a)pyrene; pulmonary neoplasia.

During life, including the period of prenatal development, the human body is exposed to many chemical substances, some of which may be potential carcinogens and tumor growth promoters. That transplacental carcinogenesis can take place in man has been demonstrated in principle by epidemiological observations on the after-effect of the use of diethylstilbestrol during pregnancy [10]. The transplacental carcinogenic activity of more than 60 substances and their combinations, including several different classes of chemical compound, has been discovered in experiments on animals of 8 species [8, 9, 11]. However, the degree of risk which these substances represent for human offspring has not yet been decided. One approach to the study of the problem of extrapolation of experimental data on transplacental carcinogenesis to man is a comparative study of the effect of carcinogens in different types of cultures of the cells, tissues, and target organs of animals and man. Organ cultures, which we have used to study transplacental carcinogenesis and as a test system for the discovery of carcinogenicity of substances [5], are most appropriate for these purposes. We have used this model to study the species- and line-specific sensitivity of the respiratory epithelium of human and animal embryos to the direct action of pneumotropic carcinogens [3]. We were interested in the possibility of assessing the effect of different doses of a carcinogen on the respiratory epithelium of animals differing in their sensitivity to spontaneous and induced pulmonary carcinogenesis.

In this paper we give the results of a morphological study of the effect of the direct action of various doses of benz(a)pyrene (BP) on embryonic lung organ cultures from mice resistant (C57Bl) and predisposed (A) to pulmonary neoplasia.

EXPERIMENTAL METHOD

Lungs of 17-day mouse embryos of lines A and C57Bl were used for organ culture. The technique of culture was described in detail previously [4]. For the first 14 days the experimental explants were grown on nutrient medium containing BP in concentrations of 3, 6, 12 μg/ml, after which they were cultured without carcinogen. Control explants were grown without the carcinogen. The cultures were studied periodically on the 7th, 14th, and 21st days of the experiment. After histological treatment serial paraffin sections, stained with hematoxylin and eosin, were studied in the light microscope. Altogether 93 control and 225 experimental embryonic lung explants from A mice and 108 control and 270 experimental embryonic lung explants from C57Bl mice were studied in ten series of experiments. The chi-square test was used for statistical analysis of the results.

EXPERIMENTAL RESULTS

In the overwhelming majority of control embryonic lung explants from mice of both lines organotypical and differentiation of the respiratory epithelium were observed with the formation of branching bronchial structures and alveolar spaces (Fig. 1A). Only in a few explants were hyperplastic changes found in the bronchial epithelium, in the form of small foci in the zone adjacent to the filter. In mice of line A these changes took place in two (2.2%) of the 93 explants, compared with in one (0.9%) of 108 explants from C57Bl mice. In the

Laboratory of Carcinogenic Substances, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Napalkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 8, pp. 205-207, August, 1988. Original article submitted March 30, 1987.

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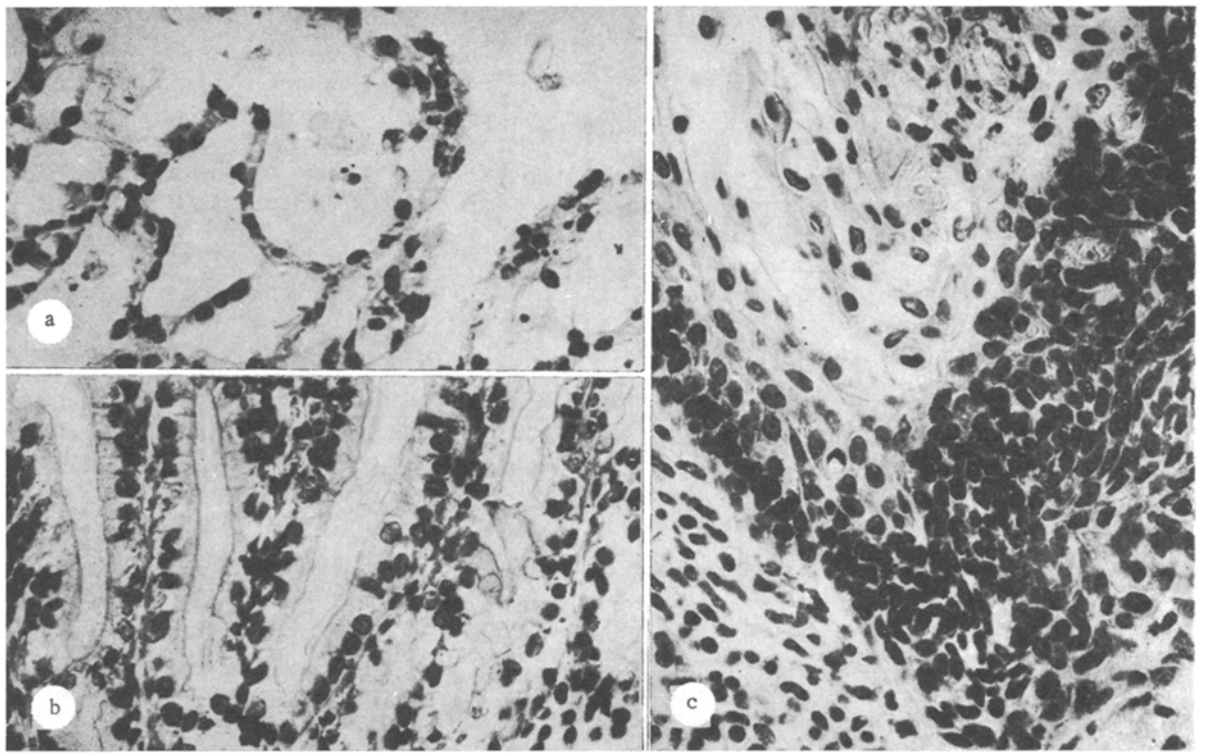

Fig. 1. Embryonic lung of line A mouse in control and experiment (14th day of culture). a) Control: organotypical differentiation of respiratory epithelium; b) BP in a dose of 6 μg/ml; papillary-adenomatous hyperplasia of epithelium; c) BP in a dose of 6 μg/ml: squamous-cell metaplasia and keratinization of bronchial epithelium. Hematoxylin and eosin. 500 ×.

early stages of culture mesenchymal cells migrated from the explants onto the surface and into the pores of the filter.

Degenerative changes devloped in the alveolar epithelium of the experimental embryonic lung explants from mice of both lines on the 7th day of culture. With doses of BP of 6 and 12 μg/ml, the regularity of the epithelial layer was disturbed in most of the alveoli, but in some alveoli and alveolar passages, the epithelial layer was virtually absent. Desquamated pycnotic cells were seen in the alveolar and bronchial cavities. During continued culture these changes gradually disappeared. BP has a toxic action on the mesenchymal cells migrating from the explants. Their number in the zone of migration was negligibly small compared with the control. On the whole, the toxic effect of BP observed in the early stages of the experiment in the embryonic lung explants from C57Bl mice was more marked than in those from A mice.

On the 14th-21st days of the experiment, against the background of residual toxic effects of BP, diffuse-focal changes in the bronchial epithelium, total papillary-adenomatous hyperplasia of the epithelium (Fig. 1b), and squamous-cell metaplasia of the bronchial epithelium with or without keratinization, often combined with basal-cell proliferation (Fig. 1c), developed in the experimental explants. The frequency and types of the morphological changes developing in the experimental embryonic lung explants on A and C57Bl mice differed. Diffuse-focal hyperplasia of the epithelium in line A mice was found after exposure to BP in doses of 3, 6, and 12 μg/ml respectively in 45.1, 56.3, and 53.2% of explants, compared with 35.5, 20.2, and 36.1% of explants from C57Bl mice (in all cases $p < 0.001$). The frequency of these changes in mice of both lines did not depend significantly on the dose of BP. However, with doses of BP of 6 and 12 μg/ml there were significant interlinear differences in the frequency of epithelial hyperplasma ($p < 0.001$ and $p < 0.01$, respectively). Squamous-cell metaplasia of the epithelium in line A mice occurred in 2.4% ($p > 0.1$), 10.4% ($p < 0.05$) and 23.4% ($p < 0.001$) of explants corresponding to the doses of BP used. It was found in only single explants from C57Bl mice (2.0-4.2%) with all doses of BP used. Total papillary-adenomatous hyperplasia of the epithelium developed only in line A mice. Its frequency increased with an increase in the dose of BP, to 10.0% ($p < 0.05$), 18.9% ($p < 0.001$), and 36.1% ($p < 0.001$).

Pulmonary carcinogenesis and, in particular, development of pulmonary adenoma in mice are characterized by definite morphological stages of development, which occur in experiments both in vivo and in vitro [1, 8]. According to Shabad's classification [7], diffuse and focal hyperplasia of epithelium are the first two stages of formation of pulmonary adenoma. It will be clear from the results described above that these stages of adenoma development, which are the least specific, occurred in experimental explants from mice of both lines quite frequently, Statistically significant differences between the two lines in the frequency of epithelial hyperplasia were found only with doses of 6 and 12 μg/ml, but within the same line, the effect on this parameter was independent of dose. Total adenomatous hyperplasia of the epithelium, the third and final stage of adenoma formation, induced in vitro [8], occurred only in mice of the sensitive A line, and its frequency increased with an increase in the dose of BP. So far as squamous-cell metaplasia of the epithelium, a feature of dysplasia of the respiratory epithelium [6], is concerned, it was found in single C57B1 explants with all doses of BP, but in explants from A mice its frequency increased significantly with an increase in dose. In other words, under organ culture conditions definite interlinear differences were found in the realization of the neoplasia-inducing effect of BP, but dose-dependence of the effect was observed only in line A mice. Similar results were obtained by the writers previously in experiments in vivo to study the transplacental effect of different doses of BP and urethane in mice of lines A and C57B1 [3].

The results of the present investigation also are evidence that in the early stages of exposure to the carcinogen it has a toxic action on the alveolar epithelium and mesenchymal cells, the intensity of the changes observed increasing with an increase in the dose of BP, and being greater in C57B1 mice. These results correlate with the results of a previous autoradiographic study [2]. Comparison of the toxic effect of BP, which predominates in the early stages of the experiment, with its carcinogenic effect, manifested later, shows that no direct correlation could be observed between sensitivity of the organs and target cells to the toxic action of the carcinogen and to its carcinogenic action, as is generally considered to be the case.

The results thus indicate that the use of organ cultures is an adequate and promising method for the comparative study of species and linear sensitivity of target organs to carcinogens and for the quantitative estimation of their action.

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