REFERENCES

1. V. V. Men'shikov (Ed.), *Laboratory Methods of Investigation in Clinical Medicine* [in Russian], Moscow (1977).
2. T. N. Ganzen, *Science and Technology Review. Series Pathological Anatomy* [in Russian], Vol. 8, Moscow (1990), pp. 123-130.
3. E. A.Neifakh, Investigation of Vitamin Systems E and F during Malignant Growth. Ph.D.Thesis, Moscow (1978).
4. G. W. Burton, K. H. Cheesman, T. Doda, *et al.*, Ciba Foundation Symposium 101: Biology of Vitamin E., London (1983), pp. 4-14.
5. H. J. Kayden, L. Hatam, M. G. Traber, *et al.*, *Blood*, **63**, № 1, 213-215 (1984).
6. F. Lindar, *Verh.Deutsch Ges. Path.*, **45**, 144-150 (1961).
7. J. Ostrowski, *Pol.Tyg.Lec.*, **34**, № 50, 1955-1959 (1979).
8. R. W. Swick and C. A. Bauman, *Cancer Res.*, **11**, № 10, 948-953 (1951).
9. S. L. Taylor, M. P. Landen, and S. L. Tappel, *Lipids*, **11**, № 7, 530-538 (1976).
10. J. N. Thompson, P. Erdody, R. Brien, *et al.*, *Biochem. Med.*, **5**, № 1, 67-89 (1971).

Benz(a)pyrene Induces Squamous-Cell Metaplasia of the Respiratory Epithelium in Explants of Embryonic Mouse Lungs

T. S. Kolesnichenko and T. G. Gor'kova

UDC 616.24−006.6−02:615.277.4:665.44.015.3−07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, No. 3, pp. 293−295, March, 1993. Original article submitted November 16, 1992.

Key Words: *organ cultures; respiratory epithelium; squamous-cell metaplasia; lung blastomogenesis*

Squamous-cell metaplasia of the respiratory epithelium occurs in the lungs of patients undergoing surgery for malignant and inflammatory diseases of the lungs and in individuals dying from other causes as well [10]. Metaplasia of the respiratory epithelium, in combination with other alterations specific to respiratory epithelium dysplasia, is most frequently found in men with a long history of heavy smoking [6]. Smoking is thought to be related to the preferential development of central lung cancer of a distinct histological type, namely, squamous-cell lung cancer typical for men [3,7,8]. The mortality from lung cancer in Russia and several other countries is increasing in parallel with the spread of the smoking habit among men and women [1,4]. On the other hand, in some countries the level of lung cancer morbidity has dropped following a decrease in the numbers of men and women smokers [8]. A drop in the frequency of squamous-cell dysplasia of the respiratory epithelium in men after quitting smoking has been noted in some reports [6]. The relationship between smoking, frequency of the development of squamous-cell respiratory epithelium metaplasia, and squamous-cell carcinoma of the lungs is consistent with the notion that squamous-cell metaplasia of the respiratory epithelium is one of the stages of development of squamous-cell lung cancer [5]. One of the components of tobacco smoke is benz(a)pyrene (BP), and its carcinogenic properties are thought to be con-

Laboratory of carcinogenic compounds, Research Institute of Carcinogenesis, Cancer Research Center, Russian Academy of Medical Sciences, Moscow. (Presented by N. N. Trapeznikov, Member of the Russian Academy of Medical Sciences)

0007−4888/93/0003−0320$12.50

nected with the development of lung cancer. We were interested in probing the possibility of inducing squamous-cell metaplasia of the respiratory epithelium by applying BP directly to the target organ. Fetal lung organ cultures were used as the model in this study. The lungs were taken from mice of the A and C57Bl/6 strains, which are, respectively, susceptible and resistant to lung blastomogenesis.

The goal of this study was to explore the dependence of the appearance of respiratory epithelium squamous-cell metaplasia on the dose of BP and the duration of its action on the target organ, as well as on the hereditary predisposition of organ donors to lung blastomogenesis.

MATERIALS AND METHODS

Seventeen-day-old fetuses of A and C57Bl/6 mice served as lung donors. Organ culture of the lung explants was performed as described elsewhere [2]. The cultures were exposed to BP in concentrations of 3, 6, and 12 μg/ml for the first 14 days, after which the explants were maintained for 7 more days in the absence of BP. In one series of trials exposure to BP (6 μg/ml) lasted 7 days, with subsequent maintenance for 10 days without carcinogen. In the control experiments the explants were grown without carcinogen during the whole time of observation. Some cultures treated with BP for 14 days, and some control cultures, were incubated with ^{3}H-thymidine, 1 μCi/ml, one day before the termination of the experiment. After histological and autoradiographic processing, serial sections of explants were stained with hematoxylin-eosin and examined under a light microscope. The results were statistically evaluated using the χ^2 test.

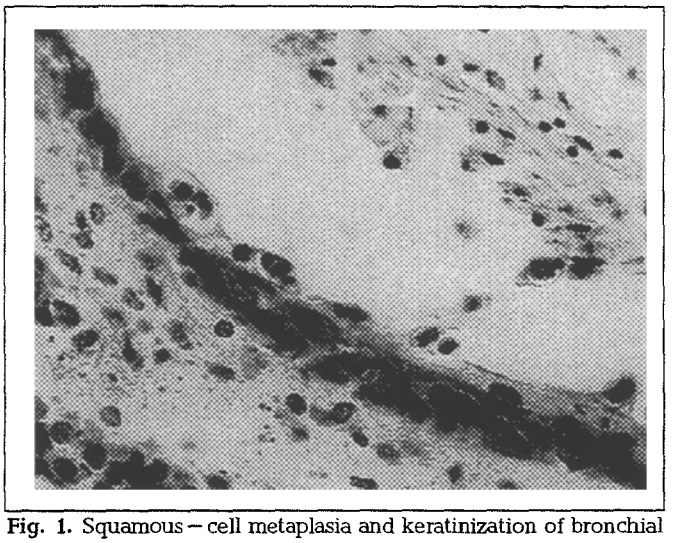

Fig. 1. Squamous-cell metaplasia and keratinization of bronchial epithelium; keratin is seen in the bronchial lumen. ×280.

RESULTS

Organotypic growth and differentiation of the respiratory epithelium, similar to the processes occurring *in situ*, were observed in the control fetal lung explants from mice of both strains. At 17-21 days the explants consisted of branched bronchial structures lined with cubical or columnar epithelium, and alveolus-like cavities formed by cubical or flattened epithelium. BP-treated explants from mice of both strains exhibited squamous-cell metaplasia of the respiratory epithelium, with or without keratinization.

TABLE 1. Frequency of BP-Induced Squamous-Cell Metaplasia of Respiratory Epithelium in Fetal Lung Explants from Mice of A and C57Bl/6 strains

Strain	BP dose, μg/ml	Duration of culturing, days	Duration of BP exposure, days	Number of explants			p
				total	with metaplasia		
					abs.	percent	
A	3	21	14	82	2	2,4	>0,1
	6	21	14	96	10	10,4	<0,05
	12	21	14	47	11	23,4	<0,01
	control	21	–	93	0	0	
	6	17	7	357	0	0	
	control	17	–	520	0	0	
C57Bl/6	3	21	14	99	3	3,0	>0,1
	6	21	14	99	2	2,0	>0,1
	12	21	14	72	3	4,2	>0,1
	control	21	–	108	0	0	
	6	17	7	568	0	0	
control	17	–	555	0	0		

Note: the experimental values are compared with the values of the corresponding control group.

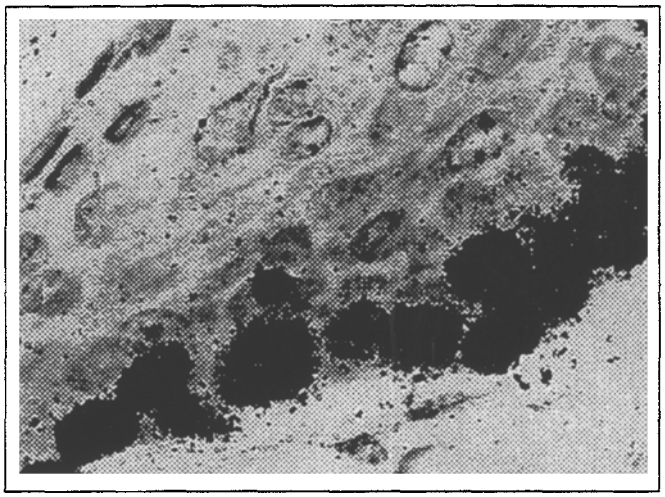

Fig. 2. Squamous-cell metaplasia and keratinization of bronchial epithelium; note intense incorporation of ^3H-thymidine in the basal cells. ×630.

Hyperplastic adenomatous outgrowths of epithelium, described earlier, [2,11], could also be seen. Squamous-cell metaplasia and keratinization of the epithelium were encountered mostly in the relatively large bronchi, the lumens of which contained keratin masses (Fig. 1). In addition, the basal epithelial cells actively incorporated ^3H-thymidine (Fig. 2). Small foci of epithelial squamous-cell metaplasia and keratinization were seen in the areas of hyperplastic epithelial proliferation. Usually, they were surrounded by atypical basophilic cells with a high nuclear-cytoplasmic ratio (Fig. 3).

The frequency of squamous-cell metaplasia development was dependent on the mouse strain, BP concentration, and the duration of exposure. For example, following a 14-day exposure (total duration of culturing 21 days) squamous-cell metaplasia appeared at all carcinogen doses used, though with varying frequency. However, a statistically significant incidence of squamous-cell metaplasia could be registered only in the explants from strain A mice and at two doses of BP: 6 μg/ml and 12 μg/ml. In these experiments a dose-dependent efect of the carcinogen was manifested (Table 1). Exposure to BP added in an effective dose (6 μg/ml) for a shorter period (7 days) failed to induce squamous-cell metaplasia of the respiratory epithelium in any explant of either A or C57Bl/6 origin.

Thus, the investigation showed that squamous-cell metaplasia of the respiratory epithelium could be induced *in vitro* by the direct action of BP in the organ culture. This corroborates the etiological role of tobacco-smoke BP in the development of squamous-cell metaplasia of the respiratory epithelium in smokers. Use of the organ culture model made it possible to prove experimentally that the frequency of metaplasia development is a function of the dose of BP and the duration of exposure of the lung tissue to it, which is in agreement with observations regarding the relationship between the incidence of this pathological state in humans and the duration and heaviness of smoking [6]. We would stress here that in our previous study [11] on organ cultures of fetal lungs from mice of the A and C57Bl/6 strains subjected to comparable doses of BP via the transplacental route, no cases of squamous-cell metaplasia of the respiratory epithelium could be found. This fact bears witness that one of the necessary conditions for the development of this pathological process is the direct action of BP on the target organ, as occurs during smoking. Use of explants from mice of strains susceptible (A) and resistant (C57Bl/6) to lung

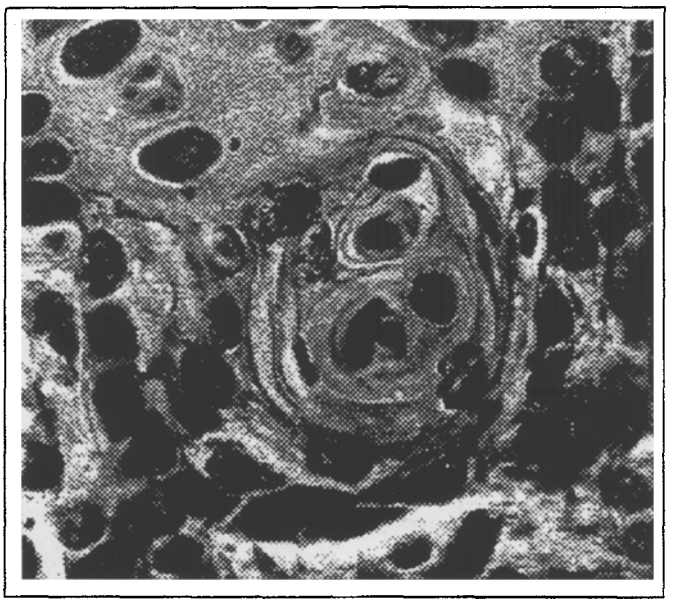

Fig. 3. Focus of squamous-cell metaplasia and keratinization of epithelium. The focus is surrounded by atypical basophilic cells with high nuclear-cytoplasmic ratio. ×630.

blastomogenesis pinpointed the role of the hereditary factor in the development of squamous-cell metaplasia of the respiratory epithelium: a statistically significant incidence of its development was recorded only in the explants of fetal lungs from mice of the cancer-prone A strain (Table 1).

Thus, we have shown the fundamental possibility of inducing squamous-cell metaplasia of the respiratory epithelium in organ cultures. It is thus possible to use tissues not only of animal but also of human origin as objects of investigation.

REFERENCES

1. R. I. Vagner, N. L. Karaseva, and E. Ya. Drukin. *Vestn Rentgenol.*, No. 1, 16-18 (1985).

2. T. G. Gor'kova and T. S. Kolesnichenko, *Byull. Eksp. Biol.*, **102**, No. 7, 69-71 (1986).
3. M. V. Dorfman, V. L. Ganul, V. L. Girko, *et al.*, *Vopr. Onkol.*, No. 12, 1469-1473 (1990).
4. B. G. Mirkin, *Analysis of Qualitative Structures and Signs* [in Russian]. p. 196-205, Moscow (1980).
5. I. G. Ol'khovskaya, *Arkh. Patol.*, No. 11, 20-25 (1985).
6. O. Auerbach, A. P. Stout, E. C. Hammond, *et al. New Engl. J. Med.*, **267**, 111-118 (1962).
7. Gao Yu-Tang, W. J. Blott, W. Zheng, *et al. Int. J. Epidemiol.*, **17**, No. 2, 277-280 (1988).
8. M. Gedes, C. Osmond, A. Barchelli, *et al. Tumori*, **71**, No. 2, 101-110 (1985).
9. Ch. R. Gillis, D. J. Holl, and P. Boyle, *J. Epidemiol. Commun. Hlth*, **42**, 38-43 (1988).
10. J. Gouveina, T. Hercend, G. Lemaigre, *et al. Lancet*, **1**, 710-712.
11. L. M. Shabad, T. S. Kolesnichenko, and T. V. Nikonova. *Neoplasma* (Bratisl.), **22**, No. 2, 113-122 (1975).

EXPERIMENTAL BIOLOGY

Stimulation of Hepatocyte Proliferation in the Normal and Pathologically Altered Liver under a Pulsed Magnetic Field

V. V. Sadovnikova, L. G. Lobko, and I. V. Sadovnikova

UDC 616.36-004-18-003:612.014.426

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 3, pp. 295-297, March, 1993
Original article submitted September 9, 1992

Key Words: *stimulation; proliferation; pulsed magnetic field; hepatitis; cirrhosis*

One of the currently studied aspects of liver regeneration is stimulation of the proliferative processes by means of various physical agents. Commonly used for this purpose are direct and pulsed currents of rectangular and exponential form and a low-frequency varying magnetic field. However, not all of these agents are sufficiently effective [6-9].

In this investigation we studied the effect of a pulsed magnetic field (PMF) with induction of 100 mT and exponential pulses lasting 1.2-3.5 msec, with a frequency of 1-10 Hz, on hepatocyte proliferation in the normal and pathologically altered liver as well as the mechanisms of cell division stimulation. In light of the fact that sympathetic nervous system transmitters play a significant role in the regulation of proliferative processes [1-5,12,14] experiments were performed to switch off the autonomic impulses by means of chemical desympathization using guanethidine and treatment with the α- and β-adrenoblockers phentolamine and anapryline.

Department of Biology, Nizhegorod Medical Institute
(Presented by I. N. Blokhin, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Proliferative activity of hepatocytes was assessed by the value of the mitotic index (MI) in parts per 1000. The following models were used: regenerating liver of normal rats (179), of rats with toxic hepatitis (128), and of rats with cirrhosis (28) induced by 70% oil solution of CCl_4 in a dose of 0.3 ml s.c. 4 times a week for 3 and 5 months.

0007-4888/93/0003-0323$12.50