POTENTIATION OF THE TOXIC EFFECT OF PULMONOTROPIC CARCINOGENS INDUCED BY DISTURBANCE OF EPITHELIAL-MESENCHYMAL RELATIONS IN EXPLANTS OF THE EMBRYONIC MOUSE RESPIRATORY TRACT

T. S. Kolesnichenko and A. L. Medvinskii

UDC 616.2-018.74-02:615.277.4.099]-092.9-07

KEY WORDS: respiratory tract; organ cultures; epithelial—mesenchymal interactions; carcinogenesis

Epithelial—mesenchymal interactions are an essential condition for prenatal ontogeny. The mesenchyme in this case has a determinant action on the development of early epithelial anlagen of various organs, especially the respiratory tract (RT). Total removal of the mesenchyme leads to cessation of morphogenesis and rapid death of the early epithelial anlage in culture, whereas preservation of, for example, one-quarter of the initial mass of the mesenchyme is sufficient to allow morphogenesis of the epithelial anlage, similar to that observed in intact explants of RT [11, 12]. The writers showed previously that disturbance of epithelial—mesenchymal interactions, caused by removal of as much of the mesenchyme as possible at the stages of organogenesis and histogenesis of RT significantly reduced the survival rate of the explants, disturbed the morphogenesis and differentiation of the respiratory epithelium (RE), and in the case of long-term culture, led to the appearance of foci of atypical growth and differentiation of cells possessing high proliferative activity [5-7]. There is evidence that the level of differentiation and proliferative activity of cells in target organs has a significant influence on sensitivity to organotropic carcinogens [8, 9]. The writers have suggested that disturbance of epithelial—mesenchymal interactions may have a modifying effect on chemical carcinogenesis. To investigate this problem experiments were carried out to study the effect of disturbance of quantitative relations between epithelium and mesenchyme on realization of the early effects of some pulmonotropic carcinogens in explants of the embryonic RT of mice predisposed (A) and resistant (C57BL) to spontaneous and induced carcinogenesis of the lungs.

This paper describes the results of experiments to study the modifying effect of disturbance of epithelial—mesenchymal relations on realization of the toxic effect of nitrosomethylurea (NMU) and benz(a)pyrene (BP) in explants of embryonic RT of mice of the above-mentioned lines.

EXPERIMENTAL METHOD

Experiments were carried out on lung explants from 17-day fetuses of A and C57BL mice. Organ culture was carried out by the method described in detail previously [1]. The effect of disturbance of the quantitative ratio of epithelium (E) and mesenchyme (M) on the rate of survival of explants after transplacental exposure to NMU and to the direct action of BP in vitro were studied. The quantitative ratio of E and M was disturbed in the following manner. Lobes of the lungs removed from the embryos were placed in a Petri dish with medium L-15, fragments of the large bronchi were removed from them, and M was taken together with them with the aid of entomological needles. Fragments of the epithelial lining of the bronchi with residues of still attached mesenchyme, obtained in this way, were explanted into organ culture. NMU was injected subcutaneously, in physiological saline in a dose of 50 mg/kg, into female mice on the 16th day of pregnancy. After 24 h the mice were killed with ether vapor and explants were prepared from the lungs of the embryos and cultured in nutrient medium not containing the carcinogen. BP was added to the nutrient medium of the cultures in a concentration of 6 μg/ml. The explants were cultured in the presence of the carcinogen for the first 14 days of the experiment, and were subsequently cultured without BP. The survival

Laboratory of Carcinogens, 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. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 8, pp. 190-192, August, 1990. Original article submitted November 27, 1989.

 0007-4888/90/0008-1100$12.50

TABLE 1. Survival Rate of Intact and Experimental Explants of Embryonic Mouse RT

Conditions	Region of RT	Line of mice			
		A		C57BL	
		number of explants			
		total	with foci of necro	total	with foci of necrosis
Intact	P	58	0(0)	61	0(0)
	D	58	1 (1,8)	57	0 (0)
NMU	P	29	0 (0), $p>0,1$	30	0 (0), $p>0,1$
	D	28	0 (0), $p>0,1$	26	0 (0), $p>0,1$
BP	P	30	0 (0), $p>0,1$	29	0 (0), $p>0,1$
	D	29	4 (13,8), $p>0,1$	28	3 (10,4), $p>0,1$
Removal of M	P	35	4 (11,4), $p>0,1$	36	1 (2,7), $p>0,1$
	D	23	9(39,1), $p<0,01$	23	4(7,3), $p>0,1$
NMU + removal of M	P	20	8 (40,0), $p<0,01$ $p_1<0,01$	20	5 (25,0), $p<0,01$ $p_1<0,01$
	D	11	6 (54,5), $p<0,01$ $p_1<0,01$	12	3 (25,0), $p<0,01$ $p_1<0,01$
BP + removal of M	P	16	10 (62,5), $p<0,01$ $p_2<0,01$	16	5 (31,3), $p<0,01$ $p_2<0,01$
	D	12	8(66,7), $p<0,01$ $p_2<0,001$	13	9 (69,2), $p<0,01$ $p_2<0,001$

Legend. P) Proximal part of RT, D) distal part of RT, p) compared with intact explants of RT, p_1) compared with explants exposed to action of NMU, p_2) compared with explants exposed to action of BP.

rate of explants of embryonic RT of the two lines of mice was studied in the next series of experiments: I) intact explants containing fragments of the large bronchi; II) similar fragments of bronchi after removal of M; III) explants of embryonic lungs subjected to the transplacental action of NMU in vivo; IV; explants of embryonic lungs subjected to the direct action of BP in vitro; V; explants of embryonic lungs subjected to the transplacental action of NMU in vivo and to removal of M; VI) explants subjected to the direct action of BP in vitro and to removal of M. The explants of all series of experiments were fixed 14 and 40 days after the beginning of culture in Bouin's fluid, dehydrated in butanols, and embedded in paraffin wax; serial sections (6 μ) were cut and stained with hematoxylin and eosin. The results of the morphological study of the explants were subjected to statistical analysis by the chi-square test.

EXPERIMENTAL RESULTS

During long-term culture all the intact embryonic lung explants from the two lines of mice remained viable. Characteristic organotypical structures were formed in them: alveolarlike cavities lined with flattened epithelium and branching bronchial structures, lined by cubical or cylindrical epithelium.

Transplacental exposure to NMU had no toxic effect on explants of the embryonic RT of mice of either line. Their survival rate was the same as that of intact explants. Direct exposure to BP led to only a very small decrease in the survival rate of the explants compared with the intact control. In the distal part of RT of both lines of mice the incidence of necrosis was higher than in the proximal RT, although the differences were not statistically significant (Table 1; $p > 0.1$).

The survival rate of explants exposed to removal of M also was reduced, but not significantly, compared with the intact control ($p > 0.1$). Only in the distal part of RT was this difference more marked and statistically significant (Table 1, $p < 0.01$).

Exposure to the combined action of carcinogens and removal of M led to a sharp decrease in survival rate of the explants of embryonic RT of both lines of mice. For instance, in the case of exposure to NMU and removal of M the frequency of foci of necrosis in the epithelium of the proximal and distal parts of RT reached 40.0-54.4% respectively in the A mice and 25.0% in the C57BL mice. After exposure to BP and removal of M the frequency of foci of necrosis in the epithelium of the proximal and distal parts of RT in the A mice reached 62.5-62.7% respectively, compared with 31.3-69.5% in the C57BL mice (Table 1).

Thus the results of experiments on the comparative study of survival of explants subjected to the factors mentioned above, or to combinations of them, showed that combined action of carcinogens and removal of M induced a sharp decrease in the survival rate of the explants compared with the intact control, and also with explante subjected to the action of carcinogens alone ($p_{1,2} < 0.01, p_2 < 0.001$, Table 1). Analysis of the results also indicates that in the case of combined exposure, what takes place is not the simple summation of the effects of each factor separately, but potentiation of the toxic effect when their action

on explants of RT is combined. Under these circumstances the toxic effect in epithelium of the distal part of RT of embryos of both lines of mice was higher in nearly all series of the experiment (Table 1) than in the proximal part of RT.

Mice of the A and C57BL lines are known to differ in their predisposition and resistance to spontaneous and induced carcinogenesis of the lungs [4]. These interlinear differences are already formed in prenatal ontogeny and are exhibited in experiments both In vivo and in vitro [10]. Under the conditions of the present experiments, interlinear differences also were exhibited in sensitivity to the toxic effect of the doses of the carcinogens used, and also to removal of M and to the combined effect of these factors. However, these differences were not statistically significant.

Thus disturbance of epithelial–mesenchymal interactions induced by partial removal of the mesenchyme significantly increases the sensitivity of the RE of mouse embryos to the toxic action of pulmonotropic carcinogens.

LITERATURE CITED

1. E. E. Antoshina and T. S. Kolesnichenko, Byull. Éksp. Biol. Med., No. 4, 119 (1982).
2. T. G. Gor'kova and T. S. Kolesnichenko, Byull. Éksp. Biol. Med., No. 11, 69 (1986).
3. T. G. Gor'kova and T. S. Kolesnichenko, Byull. Éksp. Biol. Med., No. 8, 205 (1988).
4. L. A. Gritsyute, Experimental Tumors of the Lungs [in Russian], Moscow (1975).
5. T. S. Kolesnichenko and A. L. Medvinskii, Ontogenez, **17**, No. 3, 293 (1986).
6. T. S. Kolesnichenko and A. L. Medvinskii, Ontogonez, **20**, No. 4, 381 (1989).
7. A. L. Medvinskii and T. S. Kolesnichenko, Byull. Éksp. Biol. Med., No. 7, 79 (1986).
8. A. L. Medvinskii and T. S. Kolesnichenko, Byull. Éksp. Biol. Med., No. 7, 79 (1986).
9. V. N. Anisimov, in: Modulators of Experimental Carcinogenesis, V. Turusov and R. Montesano (eds.) (IARC Sci. Publ. 51), Lyon (1983), pp. 99-113.
10. J. Ehlvest, T. Veidebaum, and E. Pöldvere, in: Modulators of Experimental Carcinogenesis, V. Tuzusov and R. Montesano (eds.) (IARC Sci. Publ. 51), Lyon (1983), pp. 295-303.
11. T. S. Kolesnichenko, in: Modulators of Experimental Carcinogenesis, V. Turusov and R. Montesano (eds.) (IARC Sci. Publ. 51), Lyon (1983), pp. 81-99.
12. J. Masters, Develop. Biol., **51**, 98 (1976).
13. B. S. Spooner and M. Wessels, J. Exp. Zool., **175**, 445 (1970).