Effect of Isobutyl Ester of Retinoic Acid on the Cellular Composition of Bronchoalveolar Lavage and on the Mitotic Activity of Alveolar Monocyte-Macrophages in Rats

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Different doses of the isobutyl ester of retinoic acid were administered intraperitoneally into rats for two weeks. The dose of 1 mg/kg caused a significant increase in the mitotic activity of cells of the macrophagal series in alveoli; an anti-inflammatory effect was observed at a dose of 10 mg/kg. The isobutyl ester of retinoic acid did not affect the cell ratio (alveolar macrophages, lymphocytes, neutrophils, eosinophils) in the internal medium of the lungs.

Key Words: *alveolar macrophages; mitotic activity; retinoids; bronchoalveolar lavage*

Alveolar macrophages (AM) are mainly replenished in two ways: due to the inflow of monocytes from the blood flow of the lungs to the alveoli and by the proliferation of AM and monocytes directly in the alveoli [5]. We demonstrated earlier that the mitotic activity of AM-monocytes increases under conditions of experimental anthracosis, as well as during compensatory growth of the lung after a left-side pulmonectomy in rats [6,7]. In this connection the possibility of pharmacological stimulation of AM-monocyte proliferation has been posited. Our attention was drawn to retinoids, which participate in the regulatory processes of proliferation, differentiation, and functioning of various cells, including the lungs [2]. Retinoids exhibit anticarcinogenic and immunomodulating properties and have the ability to suppress tumor growth [2-4]. A deficiency of vitamin A may induce bronchitis in animals, bronchopneumonia in children, and differential disturbances of the bronchial epithelium and the appearance of bronchoectasis and atelectasis in adults [10]. Ochiai [11] established a dose-dependent effect of vitamin A on the proliferation of human embryo bronchial cells in culture.

The present study was aimed at ascertaining the effect of various retinoid doses (using the isobutyl ester of retinoic acid - IERA as an example) on the cellular response in the lungs and on AM-monocyte proliferative activity.

MATERIALS AND METHODS

Forty-eight male Wistar rats weighing 130-170 g were used in the study. The IERA preparation based on soybean oil was kindly supplied by Dr. V.I.Nozdrin (Central Research Institute of Skin and Venereal Diseases, Russian Ministry of Health). IERA was administered intraperitoneally daily for two weeks at doses of 1, 10, and 100 mg/kg body weight in 0.5 ml soybean oil (each group consisted of nine animals). Intact animals (12 rats) and animals which received i.p. injections of 0.5 ml soybean oil per 100 g body weight (nine rats) served as the control. Bronchoalveolar lavage (BAL)

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TABLE 1. Main Cellular Characteristics of Rat BAL upon Administration of Different IERA Doses

Index	Intact control (n=8)	Control (soybean oil) (n=8)	IERA, mg/kg		
			1 (n=8)	10 (n=8)	100 (n=6)
Number of cells in 1 ml BAL×10^6	0.16±0.0297	0.36±0.129	0.23±0.034	0.21±0.032	0.34±0.051*
Number of AM–monocytes in 1 ml BAL×10^6	0.15±0.029	0.35±0.125	0.21±0.029	0.21±0.032	0.31±0.045*
Number of inviable cells (AM–PMNL), %	3.23±1.07	3.10±1.53	10.64±3.93*	3.02±1.43	1.22±0.60*

Note. Asterisk denotes reliable differences (p<0.05) from the control; PMNL: polymorphonuclear leukocytes. Animals with signs of lung pathology and neutrophilia of more than 20% were rejected for M±m calculation.

was obtained from Nembutal-narcotized rats in the morning without removal of the lungs after the termination of IERA administration. The number of cells in 1 ml of BAL was counted in a Goryaev chamber and cell viability was determined after staining with 1% trypan blue. Cytological preparations were made from a cell suspension of noncentrifuged BAL and a cell monolayer was obtained after the original technique of Romanova *et al.* [8]. The preparations were stained by the method of Romanowsky-Giemsa. The endopulmonary cytogram was determined by the percent ratio of different cells by analyzing 500 cells in cytological preparations, and the number of mitoses per 2000 mononuclear AM and monocytes (6-8 rats from each group) was calculated. The mitotic index was expressed in promille.

RESULTS

A two-week administration of soybean oil and IERA prepared on it was accompanied by fatty mesenterium and peritoneum viscerale at the site of the porta hepatis in all animals. This may be indicative of changes in lipid metabolism.

Different doses of IERA were found to exert different biological effects on the animals.

It has been established that 40-50% of experimental rats kept under vivarium conditions show clinical signs of respiratory diseases, and autopsies have revealed lung atelectasis and interstitial pneumonias in 40.7% of cases [1]. In 33% of cases we observed a spontaneous lung pathology (lobe infarctions and abscesses) in the lungs of 12 intact control animals. The IERA preparation at a dose of 10 mg/kg produced a pronounced anti-inflammatory effect: none of eight autopsies revealed signs of lung pathology. The incidence of spontaneous lung pathology proved to be the same (11.1%) after the administration of soybean oil and IERA at doses of 1 and 100 mg/kg.

The total number of cells in 1 ml BAL was unchanged upon administration of soybean oil and IERA in all doses studied (Table 1). Nor were significant differences observed in the cell viability index among the groups. However, the administration of IERA in a dose of 100 mg/kg was accompanied by a reliable twofold increase in both the total number of cells and the AM-monocyte number in 1 ml BAL (Table 1). Since this dose of IERA brings about an insignificant proliferation of AM-monocytes, we can assume that the increased number of cells in the internal medium of the lungs is due mainly to the inflow of cells from the microcirculatory bed to the alveoli.

Cells of the monocytic-macrophagal series were predominantly found in endopulmonary cytograms in all groups of the control and experimental ani-

TABLE 2. Degree of Development of Neutrophilic Alveolitis in Rat Lungs under Different IERA Doses

Group of animals	Number of animals	Number of animals with different degrees of BAL neutrophilia, %			
		normal (up to 3%)	moderate (3-10%)	high (10-20%)	very high (more than 20%)
Intact control	12	66.7	16.7	8.3	8.3
Control (soybean oil)	9	77.8	11.1	0	11.1
IERA, mg/kg:					
1	8	50.0	37.5	12.5	0
10	8	100	0	0	0
100	9	55.6	11.1	11.1	22.2

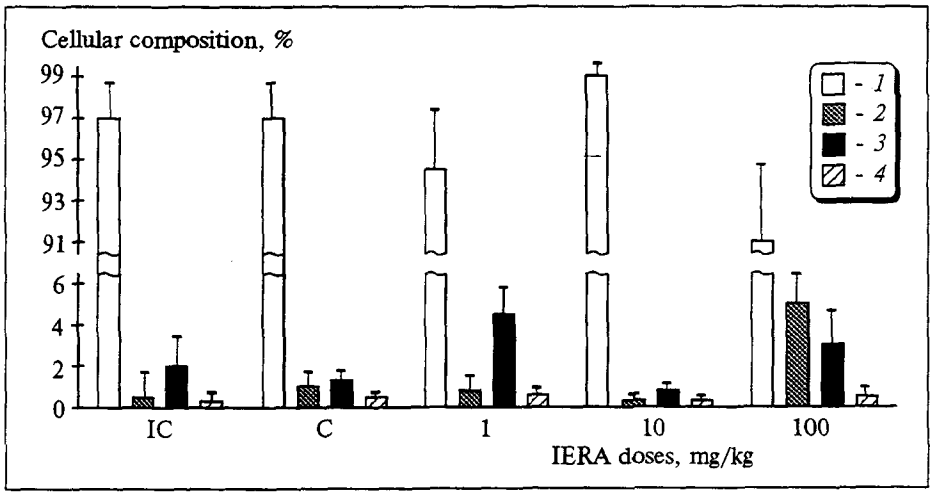

Fig. 1. Endopulmonary cytogram (ratio of different BAL cells, %). *1*) AM + monocytes; *2*) lymphocytes; *3*) neutrophils; *4*) eosinophils; *IC*: intact control; *C*: control (administration of soybean oil).

mals (Fig. 1). The composition of BAL in intact animals was as follows: 97.2% monocytes; 0.54% lymphocytes; 2.09% neutrophils, and 0.17% eosinophils. The cellular composition of BAL in all groups was similar (Fig. 1), which pointed to the absence of allergic reactions in the respiratory part of the lungs. The endopulmonary cytograms of both the control and the experimental groups showed animals with different neutrophil contents in BAL. This is a sign of either the presence or absence of the inflammatory process in the lungs manifested by neutrophilic alveolitis [9] (Table 2). A relative neutrophil content of not more than 3% in the endopulmonary cytogram attested to the absence of pronounced inflammation in the respiratory part of the lungs. IERA in a dose of 10 mg/kg evidently exerted an anti-inflammatory effect, manifested in an increased number of neutrophils in BAL in all groups of animals, which correlated with the autopsy data.

IERA in a dose of 1 mg/kg significantly stimulated ($p<0.05$) proliferation of the cells of the monocytic-macrophagal series. (The mitotic index in the experiment, intact control, and upon administration of soybean oil was 2.75±0.74, 1.0±0.15, and 1.08±0.30‰, respectively.) Changes in the mitotic index of AM-monocytes upon administration of other IERA doses (10 and 100 mg/kg) were found to be insignificant vis-a-vis the control indexes.

These results argue that IERA in all doses studied did not cause any changes in the BAL endopulmonary cytogram. Thus, administration of soybean oil and IERA in the doses indicated does not induce any allergy in the respiratory part of the lungs. IERA in a dose of 1 mg/kg stimulated proliferation of the cells of the macrophagal series in the alveoli, while a dose of 10 mg/kg had a protective anti-inflammatory effect which was manifested by the absence of inflammatory lung pathology in all experimental animals.

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