BIOPHYSICS AND BIOCHEMISTRY

Phagocytic Activity of Alveolar Macrophages in the Presence of Liposomal "Biene"

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Phagocytic activity of alveolar macrophages isolated from rat bronchoalveolar lavage fluid was studied during their reaction with multilamellar liposomes containing biene (a complex of unsaturated fatty acids). Liposomes with incorporated biene significantly stimulated phagocytic activity of freshly isolated macrophages and primary culture of these cells (judging from the increase in phagocytic index and phagocytic number). A quantitative relationship between the phagocytic number and phagocytic index, on the one hand, and the concentration of liposomal biene, on the other, was detected. The effects of liposomes containing incorporated biene, liposomes of similar composition without biene, and pure biene substance on cell suspension were compared.

Key Words: *alveolar macrophages; biene complex of unsaturated fatty acids; multilamellar liposomes; phagocytosis*

The manufacture of a series of biene-based drugs is launched at the Belmedpreparaty Company. Biene is a complex of ethyl esters of higher fatty acids obtained by microbiological synthesis from *Entomophora virulenta* mycelial fungus biomass and α-tocopherol acetate [2,3,4]. We studied the conditions of the formation of liposomal form of biene fatty acid complex used for inhalation therapy of respiratory diseases.

When using liposomes for target delivery of drugs, it is essential to have information about the effects of the pure drug substance, its liposomal form, and drug carrier (liposomes) on cell function at the earliest stage of treatment.

MATERIALS AND METHODS

The study was carried out on biene fatty acid complex (Belmedpreparaty Company), chicken egg phosphatidylcholine (Biolek Company), cholesterol (Reanal), and DL-α-tocopherol (Sigma). Multilamellar liposomes (MLL) containing fatty acid complex were formed by high-speed mechanical dispersion on a vortex [3]. Egg phosphatidylcholine and cholesterol (10:5 molar ratio) in a summary concentration of 25 mg/ml suspension were the components of liposome-forming mixture. Biene was added in a concentration of 750 μg/ml incubation mixture. Lipid peroxidation was inhibited by adding antioxidant (DL-α-tocopherol acetate) in a concentration of 1.25 mg/ml suspension.

Rat alveolar macrophages (AM) were isolated from the bronchoalveolar lavage fluid (the lungs were washed with a solution containing 140 mmol NaCl, 5 mmol KCl, 2.5 mmol phosphate buffer, 10 mmol HEPES, 6 mmol glucose, and 0.2 mmol EGTA, pH 7.4). The lavage fluid was filtered and centrifuged for 10 min at 900 rpm and 4°C. The cell precipitate was resuspended in DME (Sigma) with gentamicin and penicillin. The cell suspension was inoculated in Petri dishes in a final concentration of 3.5×10^5 macrophages/dish (Goryaev's chamber); 3 ml DME, gentamicin,

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300 µl serum were added, and the cells were incubated for 2 h at 37°C. Alveolar macrophages were isolated from the mixture of alveolar cells by their adhesion to Petri dishes; the number of adherent cells reached 98%. The liquid phase was accurately discarded, and 3 ml fresh DME (without antibiotic), 50 µl indifferent ink particles (1/200), and liposomes were added to AM. After incubation the dish was washed, the cells were fixed in methanol, stained after Romanowskii—Giemsa, and examined under an immersion microscope. The phagocytic index (percentage of phagocytic cells among macrophages) and phagocytic number (mean number of ink particles phagocytosed by one active macrophage; arb. units) [5] were evaluated.

The effect of MLL with incorporated biene on phagocytic activity of AM was studied on freshly isolated AM and on primary culture of these cells. Accordingly, liposome effects on freshly isolated AM were evaluated after 60-min incubation with liposomal biene and in parallel with this, the effects on AM primary culture were evaluated after 24-h coculturing as follows: liposomal biene (2 mg lipids/ml sample) was added to cells isolated from the same animal before AM incubation. The two cell variants were studied simultaneously because the effects caused by the liposomes in the cells can be prolonged.

In order to obtain statistically reliable data, the experiments were carried out at least 3 times. The data were processed by methods of variation statistics.

RESULTS

After 60-min incubation, MLL with incorporated biene stimulated significantly (by 76.7±6.0%) phagocytic activity of freshly isolated active macrophages in comparison with the control (no liposomes). Similar increase in phagocytic number (by 74.4±4.7%) was observed after long-term (24 h) incubation. Hence, phagocytic activity of cells increased rapidly (within 60 min of coculturing) under the effect of liposomal biene and was retained and virtually did not change later.

Phagocytic activity of AM isolated from rat lungs was studied with different concentrations of biene added to the incubation mixture. Multilamellar liposomes

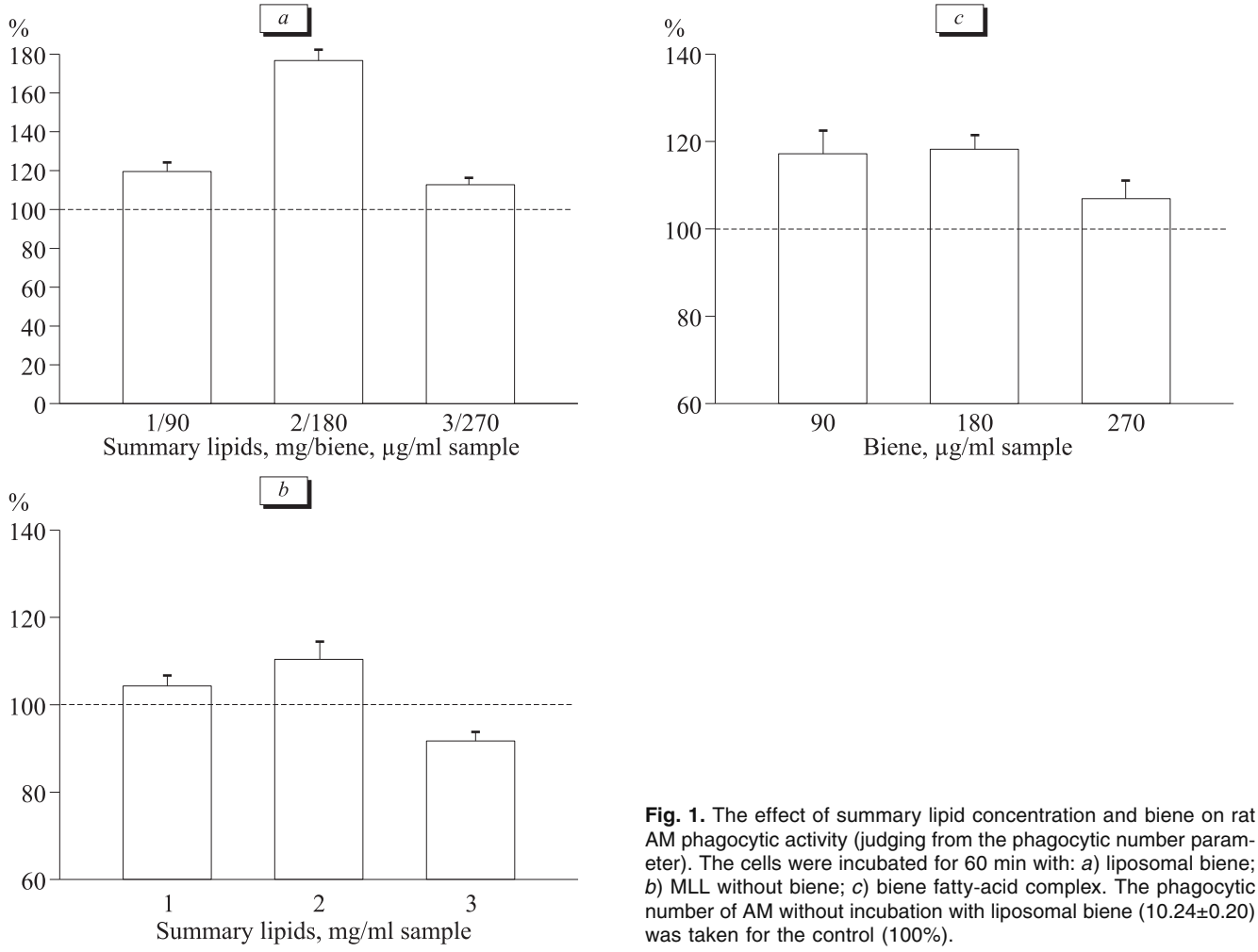

Fig. 1. The effect of summary lipid concentration and biene on rat AM phagocytic activity (judging from the phagocytic number parameter). The cells were incubated for 60 min with: *a*) liposomal biene; *b*) MLL without biene; *c*) biene fatty-acid complex. The phagocytic number of AM without incubation with liposomal biene (10.24±0.20) was taken for the control (100%).

TABLE 1. Relationship between rat AM PI and Concentrations of Liposomes and Biene Substance in Cell Incubation Medium

Samples	Lipids, mg/ml sample	Biene, μg/ml sample	PI, %
Control (AM)	—	—	93.6±1.7
AM+MLL with biene	1	90	94.4±2.1
	2	180	99.3±0.4
	3	270	92.5±1.8
AM+MLL without biene	1	—	92.3±2.7
	2	—	84.6±3.0
	3	—	84.9±1.9
AM+biene substance	—	90	92.3±1.5
	—	180	87.9±2.4
	—	270	79.9±1.1

containing the complex of unsaturated fatty acids were added to cells isolated from the same animal in concentrations of 1, 2, and 3 mg lipids/ml sample with biene content of 90, 180, and 270 μg/ml, respectively. Addition of liposomes in a concentration of 1-2 mg lipids/ml to suspension of alveolar cells increased phagocytic number by 19.5±4.5-76.7±6.0% (Fig. 1, *a*) in comparison with the control; the phagocytic index (PI) increased negligibly in this case (Table 1). A higher concentration of liposomal biene (3 mg lipids/ml; 270 μg/ml biene) also led to an increase in the phagocytic number (by 12.7±3.7%), but its absolute value was somewhat lower than after addition of MLL in concentrations of 1-2 mg lipid/ml.

Comparative analysis of the effects of MLL with incorporated biene (lipid concentrations 1, 2, 3 mg/ml sample, biene concentrations 90, 180, 270 μg/ml, respectively), liposomes of similar composition without biene (lipid concentrations 1, 2, 3 mg/ml), and biene pure substance (90, 180, 270 μg/ml) on cell suspension was carried out. Quantitative relationship between the phagocytic number and PI, on the one hand, and lipid concentration in the liposome-forming mixture and biene, on the other, was retained. Addition of "empty" liposomes and biene substance to AM led to 10-15% reduction of the total number of active macrophages (Table 1), but this effect was abolished by addition of liposomal biene to the incubation medium.

However, it is noteworthy that addition of "empty" liposomes (with lipid concentrations of 1-2 mg/ml) to incubation medium slightly (from 4.3±0.1 to 10.4±0.2%) changed AM capacity to phagocytize foreign particles (Fig. 1, *b*), while addition of MLL containing biene or the active substance alone (in concentrations of 90-180 μg/ml sample) led to a more significant increase in phagocytic activity of AM in comparison with the control. The phagocytic number increased by 20.0±0.1% (Fig. 1, *c*) after addition of biene and by 76.7±6.0% (Fig. 1, *a*) after addition of liposomes containing unsaturated fatty acids. Moreover, liposomal biene exhibited synergism of the effects of its components. It seems that this effect is explained by properties of fatty acid complex the biene.

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