Induction of Oxygen Burst in Human Blood Monocytes by Liposomes of Various Composition

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It is shown that in comparison with phorbol myristate and the calcium ionophore A23187, phosphatidylcholine liposomes less effectively induce the formation of superoxide radical in human peripheral blood monocytes. The efficiency of liposomes moderately increase after incorporation of 1,2-diacylglycerol and gangliosides, but not of esterified fatty acids.

Key Words: *liposomes; phosphatidylcholine; 1,2-diacylglycerol; human monocytes; superoxide radical*

Liposomes are widely used as a vehicle for the delivery of various drugs (antiinflammatory, antitumor, cytostatics) to tissues [6]. The main limitation to the use of liposomes is their rapid clearance from the circulation by mononuclear phagocytes [11]. The rate of phagocytosis depends on the size, composition, and surface charge of liposomes [4,5, 9] and on the surface properties of macrophages [8].

Animal experiments showed that empty (drug-free) liposomes produce considerable pharmacological effects [14], which can be potentiated by introduction of 1,2-diacylglycerol (DAG) into liposomes. It has been previously hypothesized that liposomes act through absorption by mononuclear phagocytes [7].

To elucidate the mechanisms of pharmacological effects of liposomes we studied activation of the oxygen burst in human blood monocytes incubated with phosphatidylcholine (PC) liposomes containing other lipids (DAG, fatty acid esters,. and gangliosides).

MATERIALS AND METHODS

Monocytes were isolated from donor packed white cells by centrifugation on Ficoll gradient (1.077 g/ml) at 2000 rpm for 20 min. A fraction containing B and T lymphocytes and monocytes were transferred to Petri dishes and incubated in RPMI-1640 medium (Amined) supplemented with 5% fetal calf serum in a CO_2 incubator (6% CO_2, 37°C). After 16-h incubation, the supernatant was decanted, monocyte pellet was transferred into a centrifuge tube, resuspended in incubation medium containing 15 mM phosphate buffer (pH 7.2), 10 mM glucose, and 144 mM NaCl, and centrifuged at 5000 rpm for 15 min. The pellet was resuspended in the incubation medium [15]. The cells were counted under a light microscope; 95% cell fraction were monocytes.

Liposome-induced activation of monocytes was assessed by nitroblue tetrazolium reduction. To this end, 3×10^6 cells were incubated in 1 ml incubation medium in the presence of 10 μM nitroblue tetrazolium for 10 min at 37°C and then activators or liposomes (10-100 μl) were added. After a 30-min incubation, the samples were centrifugated at 1000 rpm for 5-10 min; the supernatant was decanted, and 1 ml dioxane was added to the pellet. Optical density was measured at 520 nm in a Beckman D-6 spectrophotometer. The initial functional activity of blood macrophages was assessed by comparing reduction of nitroblue tetrazolium in the presence of phorbol myristate acetate (PMA), the Ca^{2+} ionophore A23187, and their combination.

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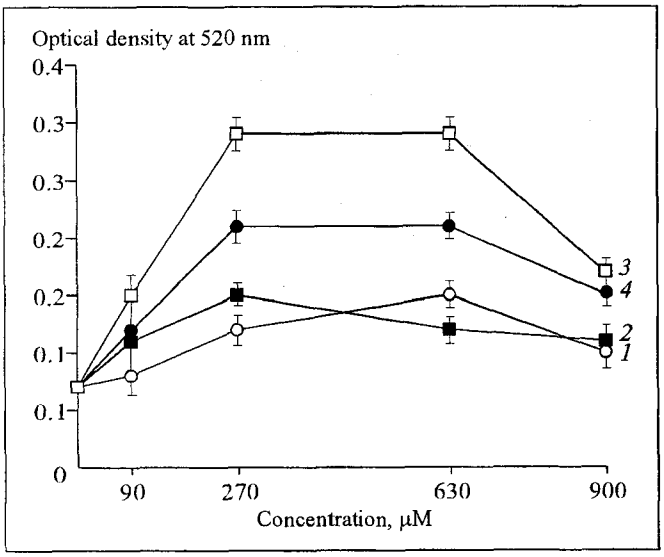

Fig. 1. Optical density of nitroblue tetrazolium incubated with human peripheral blood lymphocytes in the presence of liposomes consisting of phosphatidylcholine (PC, *1*), PC+fatty acid esters (*2*), PC+1,2-diacylglycerol (*3*), and PC+gangliosides (*4*).

For preparation of liposomes, 10% PC in ethanol (Biolek, Ukraine) was evaporated to dry and dispersed for 1-2 h in incubation medium containing 15 mM phosphate buffer (pH 7.4), 10 mM glucose, and 144 mM NaCl. Aqueous PC dispersion was sonicated for 10 min at a frequency 22 kHz and a power of 20 W with cooling (a UZDN-21 device). Liposome diameter measured by turbidimetry was 150±30 nm.

Some additional ingredients were incorporated into liposomes: DAG (95% purity, 2% of total PC) prepared from egg yolk PC by treatment with phospholipase C from *Bacillus cereus* (EC 3.1.4.3, Ferment, Lithuania), a preparation containing 40% gangliosides and 60% phospholipids from bovine spinal cord (4-8% of total PC, Biolek); fatty acid ethyl esters (2% of total PC, Biopolien, Minsk Endocrine Plant) containing palmitate (11.5%), oleate (31%), and arachidonic acid (22%) ethyl esters.

TABLE 1. Optical Density of Nitroblue Tetrazolium (520 nm) Incubated with Human Peripheral Blood Lymphocytes in the Presence and Absence of Various Activators (n=3-5, $M\pm m$, p<0.05)

Activator, μM	Optical density
Control	0.08±0.02
PMA. 0.01-1	0.16±0.02
A23187, 10	0.16±0.02
PMA, 1+A23187, 10	0.17±0.02
Liposomes, pure PC, 270	0.12±0.01
+1,2 DAG, 5.4	0.29±0.02
+gangliosides (4%), 1	0.12±0.02
+gangliosides (8%), 2	0.21±0.02
+fatty acid esters, 13.5	0.15±0.02

RESULTS

When studying oxygen bust induction, we took into account the samples characterized by a weak baseline production of superoxide radical ($O_2^{\bullet-}$, weak reduction of nitroblue tetrazolium in the absence of activators) and a surge of $O_2^{\bullet-}$ in response to PMA or A23187. Actually, 30% monocyte samples did not respond to PMA by burst production of $O_2^{\bullet-}$ and were characterized by a high initial intensity of nitroblue tetrazolium reduction, which probably attests to monocyte preactivation.

In the absence of activators, monocytes were characterized by a low intensity of $O_2^{\bullet-}$ production, and the conversion of nitroblue tetrazolium into formazan was negligible (Table 1) but this reaction considerably increased in response to PMA (0.01 μM) or the Ca^{2+} ionophore A23187 (10 mM). It should be noted that in monocytes the effect of the ionophore did not exceed that of PMA, while the effect produced by their combination was not additive, but equal to that of PMA or A23187 alone. This is probably due to the fact that A23187 in addition to increasing the intracellular Ca^{2+} concentration activates protein kinase C. Thus, both activators acted on the same target enzyme.

Incubation with PC liposomes markedly increased $O_2^{\bullet-}$ production in monocytes in a concentration-dependent manner: the effect increased within a PC concentration range of 90-270 μM and decreased above a PC concentration of 600 μM (Fig. 1). Liposomes containing fatty acid esters produced a similar effect. Introduction of DAG into liposomes did not change the shape of the concentration curve, but considerably potentiated their effect; moreover, the effect of DAG-containing liposomes surpassed that of PMA and ionophore. Addition of 4% gangliosides to liposomes did not potentiate their effect on $O_2^{\bullet-}$ production in monocytes (Table 1), while the effect of liposomes containing 8% gangliosides was similar to that produced by GAD-containing liposomes (Fig. 1).

Thus, the effect of liposomes of different composition increased in the following order:

PC=PC+fatty acid esters<PC+gangiosides<PC+DAG.

Pharmacological effect of liposomes was demonstrated not only in experimental [14] but also in clinical studies: inhalation of liposomes considerably improved the condition of asthmatic patients [2]. There are several hypothesis concerning

pharmacological effects of liposomes. It has been assumed that phospholipide liposomes injected into tissues inhibit lipid peroxidation activated in various pathological conditions [10]. Some investigators reported on antibacterial effect of lipoperoxides, obligatory admixtures in liposome preparations [1].

These findings suggest that injection of liposomes into tissue or circulation is accompanied by increased production of $O_2^{\bullet-}$, which attests to activation of human blood monocytes. These processes enhance cellular metabolism, as evidenced by accelerated surfactant production in mice after liposome inhalation [12].

The second messenger DAG incorporated into liposomes stimulates generation of $O_2^{\bullet-}$. GAD can act at different stages of the cell-liposome interaction: being a potent membrane-modifying and fusion agent [13], it promotes liposome adsorption, and, after liposome internalization, DAG activates cellular metabolism by modulating protein kinase C activity. Our findings are consistent with the data obtained on experimental animals.

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REFERENCES

1. V. M. Kreines, V. M.Mel'nikova, Ya. M. Margolin, *et al.*, *Vestn. Acad. Med. Nauk SSSR*, No. 6, 44-48 (1990).
2. K. A. Masuev, E. A. Sirotin, E. A. Limarenko, and A. G. Chuchalin, *Pulmonology*, No. 3, 68-71 (1991).
3. V. I. Trofimov and M. M. Nisevich, *Vestn. Akad. Med. Nauk SSSR*, No. 6, 28-32 (1990).
4. T. M. Allen, A. Chonn, FEBC Lett, **223**, 42-46 (1987).
5. A. Gabizon and D. Papahadjopoulos, *Biochim. Biophys. Acta*, **1103**, 94-100 (1992).
6. G. Gregoriadis, in: *Liposomes as Drug Carriers: Recent Trends and Progress*, London (1988).
7. R. Kirsh and G. Poste, *Methods Enzymol.*, **149**, 147-165 (1987).
8. K. D. Lee, K. Hong, and D. Papahadjopoulos, *Biochim. Biophys. Acta*, **1103**, 185-197 (1992).
9. D. Liu, A. Mori, and L. Huang, Ibid, **1066**, 159-165 (1991).
10. H. Nielsen, *APMIS Sect C.*, **95**, 81-85 (1987).
11. G. Poste, *Biol. Cell.*, **47**, 19-37 (1983).
12. O. A. Rosenberg, Y. A. Kirilov, L. N. Danilov, *et al.*, *J. Liposome Res.*, **4**, 203-213 (1994).
13. A. A. Selishcheva, G. M. Sorokoumova, A. S. Schragin, *et al.*, *Ibid.*, pp. 255-270.
14. T. I. Shraer, V. M. Kreines, N. A. Golubchikova, *et al.*, *Ibid.*, pp. 281-288.