EFFECT OF LIPOSOMES CONTAINING CHOLESTEROL FOR 7-KETOCHOLESTEROL FOR COLONY-FORMING ABILITY OF CELLS IN CULTURE

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Data on the connection between the cholesterol concentration in the plasma membranes of cells and their proliferative activity are somewhat contradictory [1, 3]. It has been shown [7], for instance, that when human cutaneous fibroblasts in culture pass into the resting state the total cholesterol content in the cells falls. Meanwhile there is information that the cholesterol content in the plasma membrane of certain cells is lower during their intensive proliferation than when at rest [4], and addition of cholesterol to the cell membranes leads to reduction of their proliferative activity [6]. The cytostatic action of some oxidized derivatives of cholesterol is more marked than the action of cholesterol itself [5]. It is likewise not clear which changes are primary: the proliferative activity of the cells or the cholesterol concentration in their membranes.

To compare the cytostatic action of cholesterol and its oxidized derivative, 7-ketocholesterol, their effect on proliferation of Chinese hamster cells in culture was investigated.

Suspensions of liposomes, whose ability to transport cholesterol into erythrocrte ghosts was demonstrated previously [2, 7], were used to incorporate steroid preparations into cell membranes.

EXPERIMENTAL METHOD

To prepare the liposomes we used chromatographically pure soy bean phosphatidylcholine ("Natterman, West Germany), cholesterol and 7-ketocholesterol ("Sigma," USA), dicetyl phosphate ("Serva," West Germany), and Tris ("Reanal," Hungary). The other reagents were of the chemically pure grade. Liposomes with cholesterol were prepared by the method [8] in our modification. A mixture of lipids (phosphatidylcholine/cholesterol/dicetyl phosphate in the ratio of 1:1:0.03 by weight) was dissolved in a mixture of chloroform and methanol (2:1 by volume), and the resulting solution was dried in a round-bottomed flask on a rotary evaporator for 40 min, after which medium consisting of NaCl (0.9%) and Tris-HCl (5 mM) was added to the resulting preparations. The mixture was shaken for 60 min at 37°C, after which it was treated with ultrasound (10 min) on a "Sonic-300" ultrasonic disperser ("Fisher, USA), with intensive cooling. The resulting suspension was centrifuged at 20,000*g* for 30 min. The supernatant was fertilized by filtration through a filter with pore diameter of 0.2 μ ("Nucleopor," USA). Liposomes with 7-ketocholesterol were prepared in the same way except that 25% of the cholesterol in the initial mixture was replaced by 7-ketocholesterol. It was shown by thin-layer chromatography that liposomes with cholesterol (without 7-ketocholesterol) did not contain any of its oxidized derivatives. Chinese hamster B11-dii FAR-28 cells, obtained at the Institute of Medical Genetics, Academy of Medical Sciences of the USSR, were cultured on Eagle's medium with 10% bovine serum. During testing of the preparations, cells of a 3-4-day culture (i.e., 3-4 days after seeding of the cell suspension in a Carrel flask) were removed from the glass with a mixture of standard solutions of versene and trypsin, and the suspension was diluted in growth medium and seeded on Petri dishes (about 100 cells per dish). After 2 h (i.e., after adhesion of the cells to the growth surface) the medium was poured off and solutions of the preparations in Eagle's medium without serum (1 mg/ml) were poured into the dishes. The corresponding volume of medium in which the liposomes had been prepared was added to the control dishes. After incubation of the dishes for 2.5 h at 37°C in an atmosphere with 5% CO_2,

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TABLE 1. Effect of Liposomes Containing Cholesterol Alone (LCh) or Cholesterol and 7-Ketocholesterol (LKCh) on Sufficiency of Colony Formation by Chinese Hamster Cells in Culture ("Lux" plastic dishes 90 mm in diameter, 10 ml of growth medium per dish, 6-7 dishes per preparation). Error of the Mean is Shown

Preparation	Efficiency of colony formation (relative to control), %
Control	100±6,4
LCh	9,9±2,2 ($p = 0{,}029 \cdot 10^{-4}$)*
LKCh	0

Legend. Asterisk indicates level of significance of difference from control.

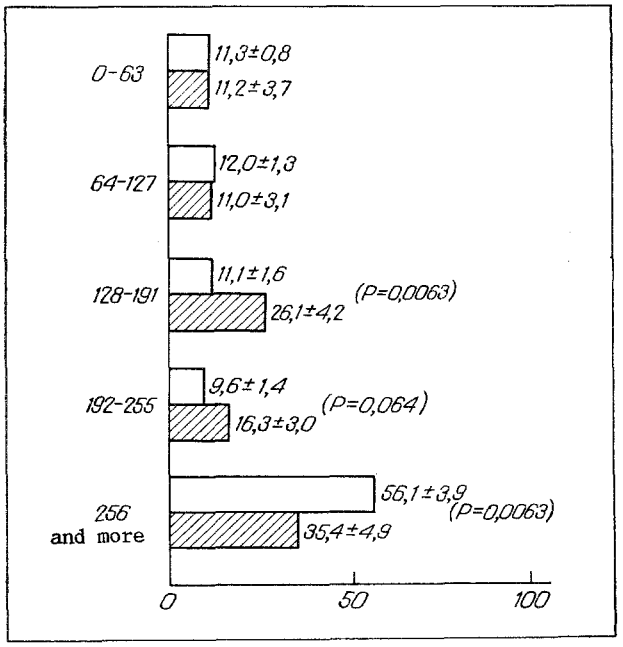

Fig. 1. Effect of liposomes with cholesterol (without 7-ketocholesterol) on distribution of colonies of Chinese hamster cells in culture by size (where differences from the control are significant, the level of significance is indicated). Abscissa, size of class (in %); ordinate, number of cells in class. Unshaded columns — control; shaded — liposomes with cholesterol.

solutions of the preparations were poured off, the dishes were rinsed with growth medium, and the necessary volume of growth medium was poured into the dishes. The dishes were then incubated at 37°C (5% CO_2) for 8 days, after which the colonies which formed were fixed with 70% alcohol and stained with a 0.1% solution of methylene blue. The number of colonies and their size (i.e., the number of cells in each colony) were then estimated in each dish.

EXPERIMENTAL RESULTS

The results are given in Table 1 and Fig. 1. Table 1 shows that under the influence of liposomes containing cholesterol only, the efficiency of colony formation by the Chinese hamster cells was reduced by 90%, whereas under the influence of preparations containing oxidized cholesterol, efficiency fell to zero (no colonies were present in the dishes).

It can be concluded from the data in Fig. 1 that under the influence of "cholesterol only" liposomes not only was the absolute number of colonies formed (i.e., the efficiency of colony formation) reduced, but there was also a decrease in the 256 and more cells" class, with a corresponding increase in the average classes ("128-191 cells" and "192-255 cells"). The dimensions of the two classes with colonies of minimal size ("0-63 cells" and "64-127 cells") remained unchanged.

Thus the addition of 7-ketocholesterol to the cell membranes completely suppressed their colony-forming ability. Incorporation of cholesterol alone into the membranes, on the other hand, had a weaker action on the proliferative capacity of the cells, although it did reduce it by 90%. Under these circumstances, judging from the altered distribution of the colonies by classes, the part of the cell population most vulnerable to the action of cholesterol was found to be the subpopulation of cells with highest proliferative activity (the "256 and more cells" class in Fig. 1), some of which, after treatment with the preparation, spilled over into the neighboring classes, characterized by a lower intensity of cell proliferation. It can be tentatively suggested that correlation between the concentration of cholesterol (or its oxidized derivatives) in the plasma membrane of the cell and its proliferative activity is a two-way connection: a change in either of these two parameters leads to a change in the other.

It can be concluded that theoretically it is possible to create lipid cytostatic preparations containing cholesterol and its oxidized derivatives for the purposes of medical practice.

LITERATURE CITED

1. O. I. Epifanova, V. V. Terskikh, and V. A. Polunovskii, Resting Cells: Properties and Functions in the Organism [in Russian], Moscow (1983).
2. A. S. Ivanov, T. S. Zakharova, and E. M. Khalilov, Biol. Membr., **5**, No. 4, 439 (1988).
3. A. N. Klimov, Current Problems in the Pathogenesis of Atherosclerosis [in Russian], Leningrad (1985), p. 26.
4. H. W. Chen, A. A. Kandutsch, and M. J. Heiniger, Prog. Exp. Tumor Res., **22**, 275 (1978).
5. D. C. Cox, K. Comai, and A. L. Goldstein, Lipids, **23**, 88 (1988).
6. M. Inbar and M. Shinitzky, Proc. Nat. Acad. Sci. USA, **71**, 2128 (1974).
7. H. S. Kruth, J. Avigan, W. Camble, and M. Vaughan, J. Cell Biol., **83**, 588 (1979).
8. V. K. Miyamoto and W. Stoeckenius, J. Membr. Biol., **4**, 252 (1971).