View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Volume 290, number 1,2, 171–172 FEBS 10190 September 1991
© 1991 Federation of European Biochemical Societies 00145793/91/$3.50
ADONIS 001457939100901Q

Effects of cholesterol- or 7-ketocholesterol-containing liposomes on colony-forming ability of cultured cells

Alexander N. Khokhlov[1], Leonid Yu. Prokhorov[1], Aleksey S. Ivanov[2] and Alexander I. Archakov[2]

[1]*Evolutionary Cytogerontology Sector, Faculty of Biology, Moscow State University, Moscow, USSR*
and [2]*Institute of Biological and Medical Chemistry, Academy of Medical Sciences, Moscow, USSR*

Received 18 July 1991

Experiments with cultured Chinese hamster cells showed that incubation of the cells with (phosphatidylcholine + cholesterol + 7-ketocholesterol)-containing liposomes (4:3:1 by weight) during two hours led to a decrease in the colony-forming ability of cells down to zero, while (phosphatidylcholine + cholesterol)-containing liposomes (1:1 by weight) reduce this parameter by 90%. Furthermore, the cholesterol-containing liposomes (without 7-ketocholesterol) induce a decrease in the number of the maximal-site colonies accompanied by the corresponding increase in the number of the middle-size colonies.

Cholesterol; 7-Ketocholesterol; Liposome; Cultured cell; Colony-forming ability

1. INTRODUCTION

There are contradictory data in some reports about the relationship of cholesterol content in cell plasma membranes and cell proliferative capacity [1,2]. In particular, it was found [3] that the total cholesterol amount of cultured human skin fibroblasts decreases during their transition to the resting state. At the same time the content of plasma membrane cholesterol in some cells during their active proliferation is lower than in the resting state [4]. The enrichment of cell membranes with cholesterol promotes a decrease in cell proliferative activity [5]. The cytostatic effect of some oxidized cholesterol derivatives is more profound than the effect of cholesterol [6]. Besides, it is not yet known which changes are primary: the cell proliferative activity changes, or the membrane cholesterol content ones. In this work we studied the action of cholesterol and its oxidized derivative – 7-ketocholesterol – on cultured Chinese hamster cell proliferation, with the aim of comparing the cytostatic effects of these compounds. For transport and introducing of sterols into cell membranes, liposomes were used. This method was tested and preparation concentrations were adjusted earlier in the experiments with cholesterol transport into erythrocyte ghosts [7].

2. MATERIALS AND METHODS

For preparation of liposomes chromatographically pure soy bean phosphatidylcholine (Nattermann, Germany), cholesterol and 7-ketocholesterol (Sigma, USA), dicethylphosphate (Serva, Germany) and Tris (Reanal, Hungary) were used. All other chemicals were of analytical grade.

Cholesterol-containing liposomes were obtained by the method described in [8] with some modifications. The lipid mixture (phosphatidylcholine/cholesterol/dicethylphosphate, 1:1:0.03 by weight) was dissolved in methanol/chloroform (2:1 by volume), dried under vacuum for 40 min and dispersed into the medium, containing NaCl (0.9%) and Tris-HCl (5 mM), pH 7.4. The mixture was shaken at 37°C for 60 min and sonicated (1 min sonication + 3 min pause) 10 times using an ultrasonic dispergator 'Sonic-300' (Fisher, USA) under cooling. The suspension obtained was centrifuged at 20 000 × *g*, for 30 min. The supernatant was sterilized by filtration through a 0.2 μm nuclear filter (Nucleopor, USA). Liposomes with 7-ketocholesterol were prepared in a similar way, but the 25% cholesterol in the initial mixture was substituted by 7-ketocholesterol. The special lipid analysis by the TLC method showed that liposomes with cholesterol (without 7-ketocholesterol) do not contain oxidized cholesterol derivatives.

Chinese hamster cells (B11-dii FAF28 line from the Institute of Medical Genetics of Academy of Medical Sciences of the USSR) were cultured on Eagle's medium with 10% of bovine serum. The cells, harvested 3–4 days after subcultivation of cell suspension in Carrel's flasks, were removed from the glass by the standard EDTA-trypsin solution. The cell suspension was diluted with growth medium and placed into Petri dishes (~100 cells/dish). After 2 h (i.e. after attachment of the cells to the growth surface) the medium was removed and the liposomal preparation, diluted with Eagle's medium without serum (1 mg of total lipid/ml), was added to the dishes. A corresponding amount of the liposome preparation medium was added into the control dishes. After 2.5 h incubation (37°C, 5% CO_2) the preparation solutions were removed, dishes were rinsed with growth medium and the required amount of growth medium was added. The dishes were incubated (37°C, 5% CO_2) during 8 days, after which the cell colonies were fixed with 70% alcohol and stained with 0.1% Methylene blue. Then the number and the size (in number of cells) of colonies were evaluated. The results obtained were analysed using the Student's *t*-test of the STATGRAPHICS program on an IBM PC/AT computer.

Correspondence address: A.N. Khokhlov, Head of Evolutionary Cytogerontology Sector, Faculty of Biology, Moscow State University, Moscow, 119899, USSR.

3. RESULTS AND DISCUSSION

The obtained data are shown in Table I and in Fig.

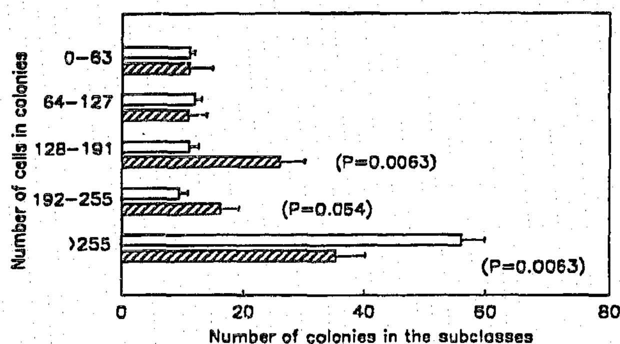

Fig. 1. Effect of the cholesterol-containing liposomes (without 7-ketocholesterol) on the colony size distribution of cultured Chinese hamster cells. In the cases of significant differences significance level is shown. Open columns, control; Hatched columns; cholesterol-containing liposomes. Results are means ± SE.

1. It can be seen that the treatment by liposomes containing cholesterol alone decreases the colony-forming ability of Chinese hamster cells by 10-fold, and the 7-ketocholesterol-containing preparation decreases it down to zero (colonies are fully absent on dishes). The liposomes containing only phosphatidylcholine did not influence the colony-forming ability.

The data in Fig. 1 show that cholesterol-containing liposomes not only decrease the absolute number of colonies appearing (i.e. colony-forming ability) but also decrease the 'greater than 255 cells' subclass, and increase the middle-size subclasses ('128–191 cells' and '192–255 cells'). The sizes of the 2 classes with the smallest colonies ('0–63 cells' and '64–127 cells') remain unchanged.

Thus, the enrichment of cell membranes with 7-ketocholesterol fully suppresses cellular ability to form colonies. However, the enrichment of membranes with cholesterol does not exert such a strong influence upon the proliferative capacity of cells; nevertheless it does decrease that capacity by 90%. The analysis of the change of colony distribution by subclasses shows that the cell subpopulation with the greatest proliferative capacity ('greater than 255 cells' class in Fig. 1) is the most sensitive to cholesterol action. After the preparation treatment, a part of this cell subpopulation moves to neighboring subclasses with the lesser cell-proliferative activity.

Table I

Effect of the liposomes containing cholesterol alone (A) or cholesterol and 7-ketocholesterol (B) on the colony-forming ability of Chinese hamster cells ('Lux' 90 mm plastic dishes, 10 ml of growth medium per dish, 6–7 dishes per preparation). SE is shown.

Preparation	Colony-forming ability (in relation to the control) %	
Control	100 ± 6.4	
A	9.9 ± 2.2	($P = 0.029 \cdot 10^{-4}$)*
B	0	

*Significance level of difference from the control

We suppose that the relationship between cholesterol content (or its oxidized derivatives) in cell plasma membranes, and cell proliferative capacity is one of interdependence: changes in either of these two parameters entail changes in the other one.

Our results (together with our earlier data concerning the effect of 7-ketocholesterol on the kinetics of proliferation of *Acholeplasma laidlawii* and Chinese hamster cells [9]) allow us to come to the conclusion that it is theoretically possible to create lipid cytostatic drugs containing cholesterol and its oxidized derivatives for practical medical use.

REFERENCES

[1] Epifanova, O.I., Terskikh, V.V. and Polunovsky, V.A. (1983) Resting Cells, Properties and Functions in Organism, Nauka Publishers, Moscow (in Russian).

[2] Klimov, A.N. (1985) in: Actual Problems of Atherosclerosis Pathogenesis, pp. 26–47, Leningrad (in Russian).

[3] Kruth, H.S., Avigan, J., Gamble, W. and Vaughan, M. (1979) J. Cell Biol. 83, 588–594.

[4] Chen, H.W., Kandutsch, A.A. and Heiniger, H.J. (1978) Progr. Exp. Tumor Res. 22, 275–316.

[5] Inbar, M. and Shinitzky, M. (1974) Proc. Natl. Acad. Sci. USA 71, 2128–2130.

[6] Cox, D.C., Comai, K. and Goldstein, A.L. (1988) Lipids 23, 85–88.

[7] Ivanov, A.S., Zakharova, T.S., Beriozov, A.T., Khalilov, E.M., Torkhovskaya, T.I. and Archakov, A.I. (1989) in: Phosphatidylcholine (Polyenephosphatidylcholine/PPC): Effects on Cell Membranes and Transport of Cholesterol (Archakov, A.I. and Gundermann, K.-J. eds) pp. 87–97, wbn-Verlag, Bingen/Rhein.

[8] Miyamoto, V.K. and Stoeckenius, W. (1971) J. Membr. Biol. 4, 252–269.

[9] Beriozov, A.T., Ivanov, A.S., Ivkov, V.G., Obraztsov, V.V., Khalilov, E.M. and Archakov, A.I. (1990) FEBS Lett. 266, 72–74.