ANDRZEJ GARDAS

STIMULATION OF DNA SYNTHESIS IN SERUM-STARVED NIL-8 CELLS
BY UNILAMELLAR LIPOSOMES*

Medical Centre of Postgraduate Education
Marymoncka 99; 01-813 Warsaw, Poland

Unilamellar liposomes added to serum-starved NIL-8 hamster fibroblasts stimulate DNA synthesis in 18 to 46% of the population. Stimulation of DNA synthesis depends on the type and composition of liposomes. The highest rate of stimulation was observed on addition of 50 μg/ml of unilamellar liposomes composed of lecithin, cholesterol and phosphatidic acid at the 10:5:1 ratio. Multilamellar liposomes added at the same concentration did not stimulate DNA synthesis.

Liposomes induce cell fusion (Papahadjopoulos et al. 1973), inhibit adenylyl cyclase (Klein et al., 1978) and stimulate differentiation of murine neuroblastome cells (Chen et al., 1976). They have been used recently to introduce into cells substances of low or high molecular mass (Gardas & Macpherson, 1979; Gregoriades, 1976; Papahadjopoulos et al., 1976a). We report here that unilamellar liposomes can stimulate DNA synthesis in the serum-starved NIL-8 cells.

MATERIAL AND METHODS

Liposomes were prepared by the method of Batzri & Korn (1973). Ethanol solution of a lipid mixture composed of egg lecithin, cholesterol and phosphatidic acid (10:5:1, by weight) was added to Dulbecco’s modification of Eagle’s medium without calcium and magnesium ions, with constant stirring under nitrogen. The final concentration of ethanol was 7%. Liposomes were collected by centrifugation at 160,000 g for 1 h and the pellet was suspended in the same medium to obtain a final lipid concentration of 1 mg/ml. The liposome suspension was sterilized by filtration through a 0.22 μm millipore filter and stored at 4°C.

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Multilamellar liposomes were prepared as follows: 5 mg of a lipid mixture consisting of lecithin, cholesterol and phosphatidic acid (10:5:1, by weight) was evaporated to dryness, then 2 ml of Dulbecco's modification of Eagle's medium without calcium and magnesium ions was added, mixed, and sonicated for 1 min. The liposomes were then collected by centrifugation at 45000 g for 1 h, washed twice with saline and stored at 4°C at a concentration of 1 mg/ml.

Lecithin was prepared from egg yolk as described by Pangborn (1951) and purified on a silicic acid column as described by Rouser et al. (1963). The purity of the lecithin was checked by thin-layer chromatography as described by Skipski et al. (1962). Lecithin was stored in ethanol solution (1 mg/ml) under nitrogen at -20°C. Phosphatidic acid was obtained from Koch Light Ltd. (Colnbrook, England), cholesterol from Sigma (St. Louis, U.S.A.), and radiochemicals from The Radiochemical Centre (Amersham, England). Uridine transport was estimated as described by Rosengurt & Stein (1977).

NIL-8 hamster cells were grown in plastic trays in 1.5 cm² wells in Dulbecco's modification of Eagle's medium supplemented with 10% calf serum; 5 x 10⁴ cells were seeded per well in 1 ml of medium and left for 24 h at 37°C. This medium was replaced with 1 ml of the medium containing 0.2% calf serum, and cells were incubated in an atmosphere supplemented with 15% CO₂ at 37°C. A week later 20 to 500 μg of liposomes and 1 μCi of [³H]thymidine were added. After 48 h of incubation the medium was removed, cells were washed with cold saline, cold 5% trichloroacetic acid and 96% ethanol. Fixed cells were covered by Ilford G5 emulsion and after one week's exposure the preparations were developed and stained with Giemsa. The percentage of cells which incorporated tritiated thymidine was assessed by counting 1000 cells in arbitrarily chosen fields.

RESULTS AND DISCUSSION

NIL-8 cells survived well at liposome concentrations below 500 μg/ml of medium. There was no difference in the number of cells which incorporated tritiated thymidine in cultures with or without liposomes when, after 48 h incubation with liposomes, they were incubated for another 24 h with 10% calf serum. At higher liposome concentrations cells started to detach from the substratum and died.

When unilamellar liposomes were added to cells starved for 7 days in serum-depleted medium, 18 to 46% of the cell populations were stimulated into DNA synthesis (Table 1). The extent of stimulation was dependent on the type of liposomes used; lower stimulation was obtained with liposomes prepared from lecithin and higher when liposomes with cholesterol and phosphatidic acid were used. Stimulation of DNA synthesis was diminished by 5% in the presence of 5 x 10⁻⁵ M-colchicine (not shown). Maximal stimulation was obtained at a liposomes concentration of 50 μg/ml, and raising the concentration of liposomes to 500 μg/ml did not increase the cell population active in DNA synthesis. Liposomes stored for a few days at 4°C stimulated DNA synthesis to a 5 to 10% higher level than
did freshly prepared liposomes. All three types of unilamellar liposomes behave in the same way as described above. This might be due to fusion of liposomes during storage into bigger vesicles which may be more efficient in stimulation of DNA synthesis. After 48 h of incubation with liposomes some cell fusion was observed. Multilamellar liposomes at the same concentration do not stimulate DNA synthesis in the serum-starved NIL-8 cells. Growth of normal cells is dependent on serum, and “serum factors” are required for initiation of DNA synthesis and for continued growth (Holley, 1975; Temin, 1966). The earliest changes that have been detected following growth stimulation were changes in the intracellular concentration of cyclic nucleotides (Otten et al., 1972; Seifert & Rudland, 1974) and changes in transport of nutrients (Hoffman et al., 1973; Pardee, 1974). Participation of calcium ions in initiation of DNA synthesis has been implicated by several authors (Balk, 1971; Dulbecco & Elkington, 1975).

**Table 1**

**Stimulation by liposomes of DNA synthesis in serum-starved NIL-8 cells**

Each figure represents the mean value from 10 experiments (±S.D.).

<table>
<thead>
<tr>
<th>Type of liposomes</th>
<th>% of cells which incorporated [H]thymidine</th>
</tr>
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<tbody>
<tr>
<td>Liposomes from lecithin: cholesterol: phosphatic acid at the ratio 10:5:1</td>
<td>46±4</td>
</tr>
<tr>
<td>Liposomes from lecithin: cholesterol at the ratio 10:5</td>
<td>31±3</td>
</tr>
<tr>
<td>Liposomes prepared from lecithin</td>
<td>18±3</td>
</tr>
<tr>
<td>Control cells</td>
<td>6±2</td>
</tr>
<tr>
<td>Liposomes from lecithin: cholesterol: phosphatic acid at the ratio 10:5:1; after 48h of incubation with liposomes, 10% calf serum was added and left for another 24h</td>
<td>95±5</td>
</tr>
<tr>
<td>Multilamellar liposomes from lecithin: cholesterol: phosphatic acid at the ratio 10:5:1</td>
<td>10±2</td>
</tr>
</tbody>
</table>

Liposomes added to the cells arrested in growth by serum starvation can initiate DNA synthesis, because they can fuse with cells and possibly introduce constituents of the medium into cytoplasm. The presence of calcium ions is necessary for cell fusion and for liposome fusion with cells. Liposomes added to medium which contains calcium ions also fuse with one another and form large vesicles (Papahadjopoulos et al., 1976b) which contain calcium ions and nutrients.

Fusion of large numbers of liposomes with cell membranes can also cause alterations in membrane structure and permeability to nutrients. However, in preliminary experiments we did not observe any drastic changes in uridine transport after addition of liposomes to the serum-starved NIL-8 cells.
REFERENCES


STYMULACJA SYNTEZY DNA PRZEZ JEDNOWARSTWOWE LIPOSOMY W KOMÓRKACH NIL-8 "WYGŁODZONYCH" PRZEZ Hodowlię W Pożywce O NISKIEJ ZAWARTOŚCI SUROWICY

Streszczenie

Jednowarstwowe liposomy dodane do hodowli komórek NIL-8, których wzrost został zahamowany przez hodowlię w pożywce o niskiej zawartości surowicy (0.2%), stymulują syntezę DNA w 16 do 46% populacji komórek. Stymulacja syntezy DNA zależy od typu i składu liposomów. Najwyższą stymulację obserwowano, gdy do hodowli komórek NIL-8 dodano 50 μg/ml jednowarstwowych liposomów o składzie lecytyna, cholesterol i kwas fosfatydowy w stosunku 10:5:1. Wielowarstwowe liposomy w tych samych warunkach nie powodowały stymulacji syntezy DNA.

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