NEW BIOMEDICAL TECHNOLOGIES

Electron Microscopic Examination of the Interaction between Human Erythrocytes and Liposomes Containing DC-Cholesterol

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 7, pp. 83-86, July, 1996
Original article submitted March 16, 1995

Human erythrocytes treated with cationic liposomes containing DOPC/DC cholesterol (2:3 mol/mol) or DOPE/DC cholesterol (2:3 mol/mol) are studied by electron microcopy. DOPE-containing liposomes exhibit a markedly greater ability to interact with the erythrocyte surface and to initiate a mosaic structure represented by smooth and rough surfaces on freeze-fractures of the erythrocyte plasma membrane. The interaction between DOPE/DC-cholesterol-containing liposomes and the plasma membrane provides the basis for using these liposomes to deliver DNA into the cell cytoplasm.

Key Words: *human erythrocytes; cationic lipids; DNA transport, membrane ultrastructure*

Liposomes containing cationic lipids are an effective means of DNA transport employed for transfection [5,6]. 3[N-(N',N'-Dimethylaminoethane)-carbamoyl]-cholesterol (DC-cholesterol) was shown to be an effective DNA transporter [6]. Generally, liposomes used for DNA transport consist of cationic and helper lipids. Dioleoylphosphatidyl ethanolamine (DOPE) was found to possess the best helper properties [5,6]. In the present study we compared the interaction of effective and noneffective transport liposomes with the erythrocyte's plasma membrane. DOPE and dioleoylphosphatidyl choline (DOPC) were used as helper lipids in effective and noneffective liposomes, respectively.

MATERIALS AND METHODS

DOPC and DOPE were obtained from Avanti Polar Lipids. DC-cholesterol was synthesized as described previously [6]. Liposomes containing phospholipid and DC-cholesterol (2:3) were prepared by ultrasound sonication in an argon atmosphere using a USDN-2T installation until a lipid concentration of 5 mg/ml was achieved. Isolation and electron microscopy of liposome-treated erythrocytes were described elsewhere [2,3]. Erythrocytes were isolated from freshly collected donor blood by triple precipitation with a mixture containing 140 mM NaCl, 5 mM KCl, 10 mM HEPES (pH 7.4). The erythrocyte suspension containing 2×10^9 cells/ml was incubated at 37°C with two volumes of liposome preparation. The incubation period was varied from 2 min to 1 h. Ultrathin sections were prepared after successive fixation with glutaraldehyde, OsO_4, tannic acid, and OsO_4 again. Freeze-fractures were prepared after fast cryofixation in overcooled propane. The replicas were shadowed with a platinum-carbon mixture.

RESULTS

Ultrathin sections of erythrocytes treated with cationic liposomes containing DOPC or DOPE are shown in Fig. 1. DOPE-containing liposomes are

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0007-4888/96/0007-0724$15.00

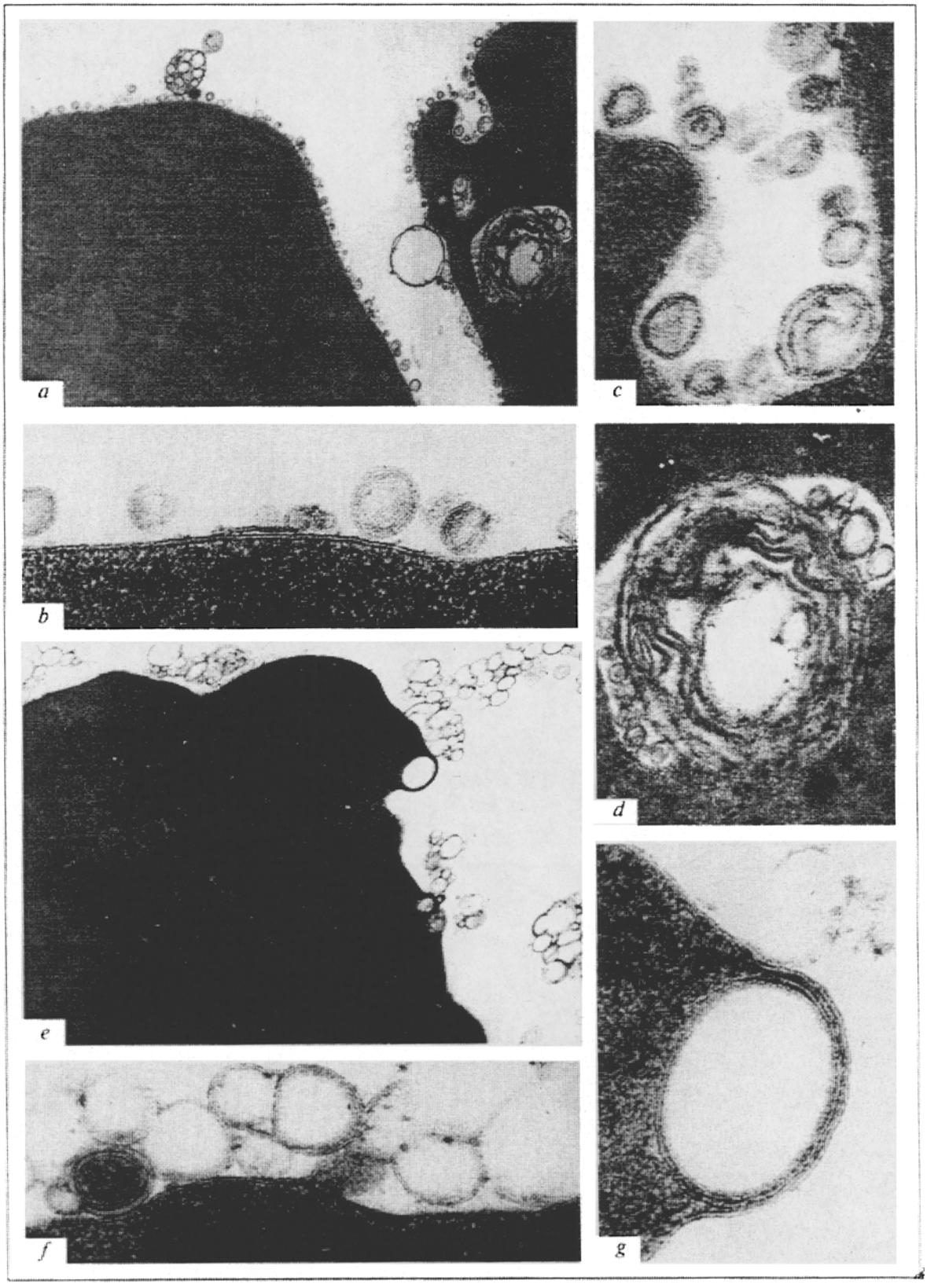

Fig. 1. Ultrathin sections of human erythrocytes treated with liposomes containing DOPC and DC-cholesterol (*a*, *b*, *c*, *d*) or DOPE and DC-cholesterol (*e*, *f*, *g*) at a molar lipid ratio of 2:3. Erythrocytes were incubated with the liposomes at 37°C for 1 h. *a*) and *e*) × 22,000; *b*), *c*), *f*), and *g*) × 140,000; *d*) × 80,000.

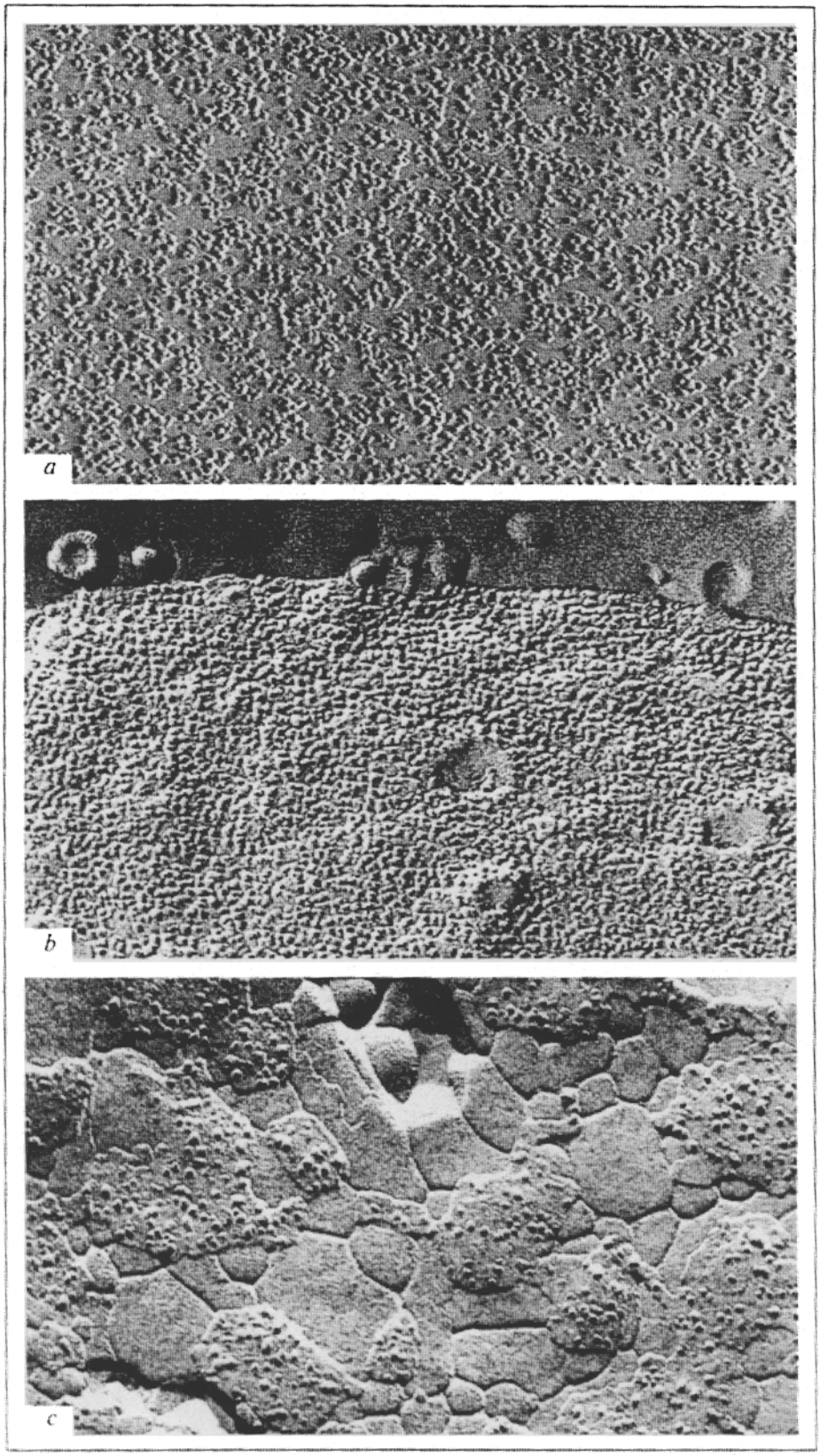

Fig. 2. Replicas from freeze-fractures of human erythrocyte plasma membrane. *a*) intact erythrocytes; *b*) erythrocytes treated with liposomes containing DOPC and DC-cholesterol; *c*) erythrocytes treated with liposomes containing DOPE and DC-cholesterol. Erythrocytes were incubated with liposomes at 37°C for 1 h. × 110,000.

considerably larger than DOPC-containing liposomes and tend to form large aggregates on the erythrocyte surface. By contrast, DOPC-containing liposomes are evenly distributed over the erythrocyte surface and form no aggregates. The interaction between the liposomes and erythrocytes manifests itself only in the formation of membrane contacts (Fig. 1, *b*, *f*). We observed no membrane fusions or formation of the structures preceding it. This indicates that the ability of liposomes containing DC-cholesterol to fuse with the plasma membrane is much weaker than that of liposomes with octadecylamine [2,3]. Liposome-treated erythrocytes tended to form plasma membrane invaginations (Fig. 1, *c*) and vesicular structures in the cytoplasm (Fig. 1, *d*, *g*), which is probably associated with processes similar to endocytosis [2,3].

A marked drop in the number of membrane particles and formation of smooth surfaces were observed on freeze-fractures of membranes contacting with liposomes (Fig. 2, *b*, *c*), which is indicative of specific interaction between the contacting membranes. This effect was more pronounced in erythrocytes treated with DOPE-containing liposomes: the surface of freeze-fracture looked mosaic with alternating smooth and rough zones (Fig. 2, *c*). By contrast, DOPC-containing liposomes initiated the formation of very small smooth zones (Fig. 2, *b*). The disappearance of membrane particles from the contact area is known to precede membrane fusion. However, membrane fusion is quite a rare event difficult to pinpoint on ultrathin sections.

We believe that a direct exchange of material between contacting membranes of cell and liposome is possible, resulting in the insertion of an exogenous lipid into the plasma membrane. In this case DNA molecules can be transported into the cell, since an inverted nonbilayer phase is formed by lipids [1,4]. The ability of DOPE-containing liposomes to actively modify the freeze-fracture of the plasma membrane correlates with the high effectiveness of DNA transport provided by these liposomes.

The study was financially supported by the Russian Foundation for Basic Research, project code 93-04-22040.

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