Comparative Study of the *In Vitro* Effect of Nanoliposomes with Oxidized Dextrans on Peritoneal Cells

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We studied the *in vitro* effect of hybrid molecular-nanosomal biocompatible compositions on cultured peritoneal cells. The compositions consisted of oxidized dextrans with a mean molecular weight of 35 and 60 kDa, which were obtained by chemical and radiochemical oxidation of dextran. Hybrid nanoliposomal compositions of chemically oxidized dextran (permanganate method) had greater biocompatibility and tropic activity to macrophages compared to nanoliposomes of radiochemically oxidized dextran.

Key Words: *oxidized dextrans; nanoliposomes; biocompatibility; peritoneal cells; macrophages*

Polysaccharides are biocompatible compounds modulating the state of cells in the mononuclear phagocyte system, which mediates organism's response to external influences [5]. Hence, they hold much promise as a biodegradable matrix for directed transport of drugs into the cell. Incorporation of these compounds into nanoliposomes (NL) can provide greater specificity of targeted transport to cells of the mononuclear phagocyte system. *In vitro* study should be performed to compare some properties (*e.g.*, biocompatibility) of NL with dextrans oxidized by various methods.

The biocompatibility of NL was *in vitro* studied on cultured peritoneal cells (PC). They consisted of oxidized dextrans (OD) with the mean molecular weights of 35 and 60 kDa, which were obtained by radiochemical (OD_r) and chemical oxidation (permanganate method, OD_c).

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MATERIALS AND METHODS

Aqueous solutions (10%) of dextran (mean molecular weights 35 and 60 kDa, Fluka) were heated on a boiling water bath at 100°C. Aqueous solution of acetic acid (33%, 2 vol % of dextran solution) was added to the solutions of dextran and agitated. Aqueous solution of potassium permanganate (2%, 4 vol % of dextran solution) was added. The mixture was heated at 100°C until precipitation. The solution was filtered through a paper filter (Enderol filter No. 3, Binzer). Oxidized dextrans were designated according to the mean molecular weight (D-ch-35 and D-ch-60). They were precipitated from the solution by adding a 2-fold volume of 95% ethanol under heating of the mixture to 60°C.

Aqueous solutions (10%) of dextrans with the mean molecular weights of 35 and 60 kDa (Fluka) were irradiated with an accelerated electron flow. The total absorbed dose was 30 kGy. They were designated according to the mean molecular weight (D-r-35 and D-r-60) [4].

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To obtain NL with OD, 200 mg phosphatidylcholine (Sigma) were added to 20 ml solution of D-ch-35, D-ch-60, D-r-35, and D-r-60 (2.5% solutions in 0.01 M Tris-phosphate buffer, pH 7.3). The process of swelling was conducted at 4-6°C for 24 h. These solutions were thoroughly agitated until the formation of a homogenous lipid suspension. The suspension was subsequently passed through acetate-cellulose filters with pore diameters of 0.8, 0.45, and 0.2 μ (Sartorius) to separate nanosomal liposomes. This treatment allowed us to obtain NL of 150-200, 200-450, and 450-800 nm.

The biocompatibility of OD-containing NL was assayed on peritoneal transudate cells of male BALB/c mice aging 2 months, weighing 21-22 g, and obtained from the nursery of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences, Novosibirsk). PC were obtained from the animals killed by cervical dislocation under ether anesthesia [2]. The biocompatibility of OD-containing NL was evaluated from their effect on adhesion activity and viability of PC (no phenotypic differentiation) [1]. Biotropic activity of OD-containing NL was estimated from phagocytosis by peritoneal macrophages. OD-containing NL were added to cultured cells by the 2nd hour of culturing. The test samples were diluted with a buffer (pH 7.3, 1:100). Cultured cells were assayed 24 h after addition of OD-containing NL to the culture medium. PC were cultured on coverslips (10^6 cells per 2 ml medium 199 with 10% fetal bovine serum) in glass flasks at 37°C. Adhesion activity of PC was estimated from the number of adherent cells, which remained after washing of cultured cells with the culture medium. The mean number of adherent cells was calculated per field of view (×200). Cell viability (ratio of living PC) in cultures was evaluated by vital staining with trypan blue [2].

By the end culturing, cultured cells were fixed with 4% formaldehyde in phosphate-buffered saline (pH 7.4, 10 min) and stained with Sudan black B (0.25% solution in 70% aqueous ethanol, 10 min) to detect NL in cells. They were washed with distilled water and subjected to safranin staining (1%

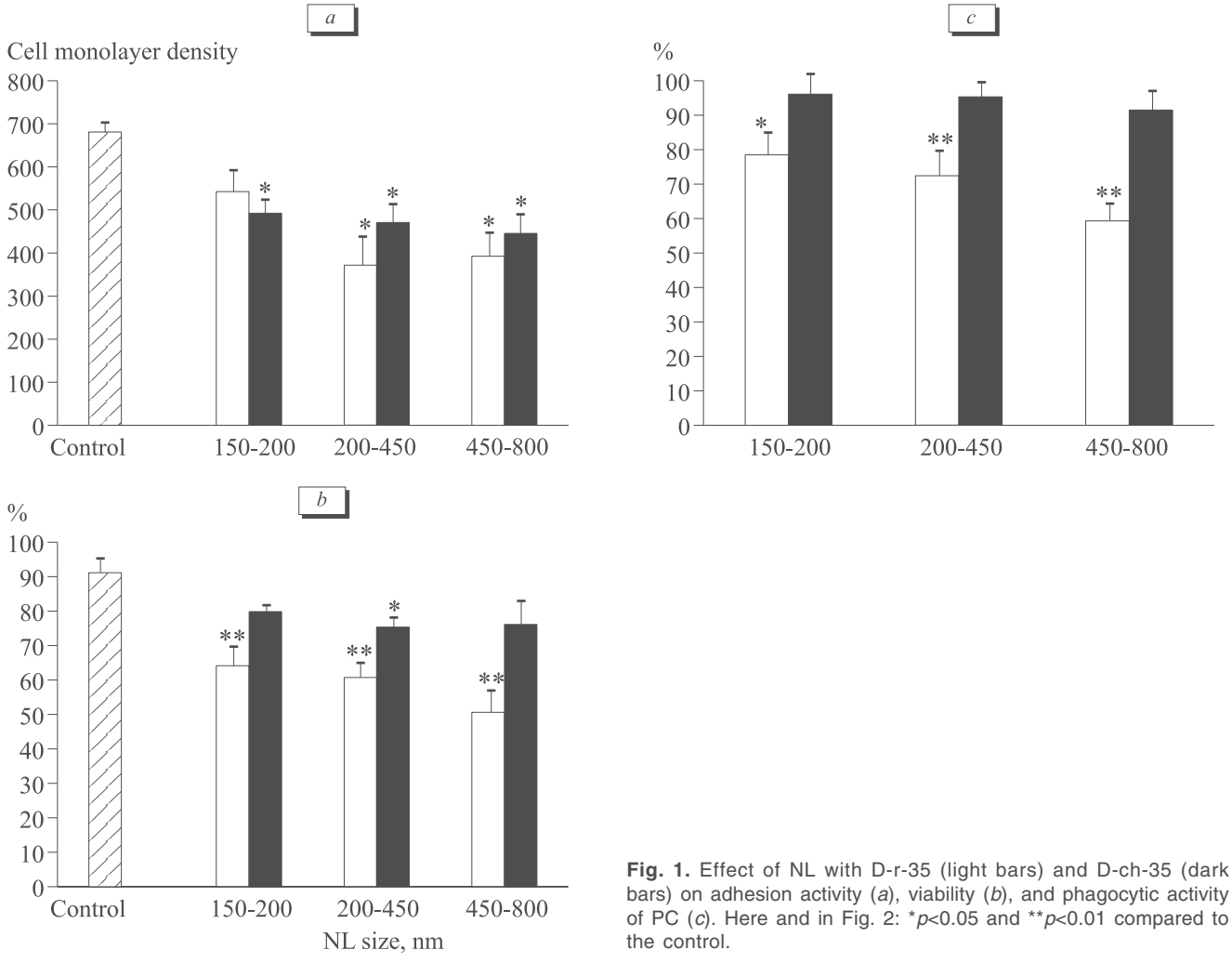

Fig. 1. Effect of NL with D-r-35 (light bars) and D-ch-35 (dark bars) on adhesion activity (*a*), viability (*b*), and phagocytic activity of PC (*c*). Here and in Fig. 2: *$p<0.05$ and **$p<0.01$ compared to the control.

aqueous solution, 10 min). Cytomorphological analysis of cultured cells was performed under an Axio-Imager Z1 microscope (Zeiss, ×100). We calculated the ratio of macrophages which phagocytized OD-containing NL (5 fields of view in each sample for test parameters; 5 cell cultures from 5 mice for each parameter).

Intact cultures of PC served as the control. The significance of differences between the mean values was estimated by nonparametric White test.

RESULTS

The majority of D-ch-35-containing NL of all sizes added to cultured cells reduced adhesion activity of PC (density of the cell monolayer; Fig. 1, *a*). This parameter did not decrease under the influence of 450-800-nm NL with D-ch-60 (Figs. 1 and 2).

When studying the effect of OD-containing NL on PC viability in the culture, we found that addition of D-ch-35- and D-r-35-containing NL of all sizes to the culture medium was followed by a decrease in cell viability. It was most pronounced during culturing with D-r-35-containing NL of all sizes (Fig. 1). Similar changes were observed after addition of D-ch-60- and D-r-60-containing NL of 200-450 nm to the culture medium (Fig. 2).

Our results indicate that the biocompatibility of OD_r-containing NL is slightly lower than that of OD_c-containing NL. OD_r probably contains cytotoxic microadmixtures of peroxide compounds that are formed during radiolysis of aqueous dextran solutions [3].

The most pronounced differences in the biocompatibility of NL with OD_c and OD_r were recealed during studying of phagocytic activity of macrophages relative to NL samples (Figs. 1 and 2). Phagocytic activity of macrophages to OD_r-containing NL was lower compared to that observed in experiments with D-ch-35- and D-ch-60-containing NL (Figs. 1 and 2). The observed differences probably result from changes in lability of the macrophage plasma membrane due to contact with the NL membrane, which carries peroxide compounds

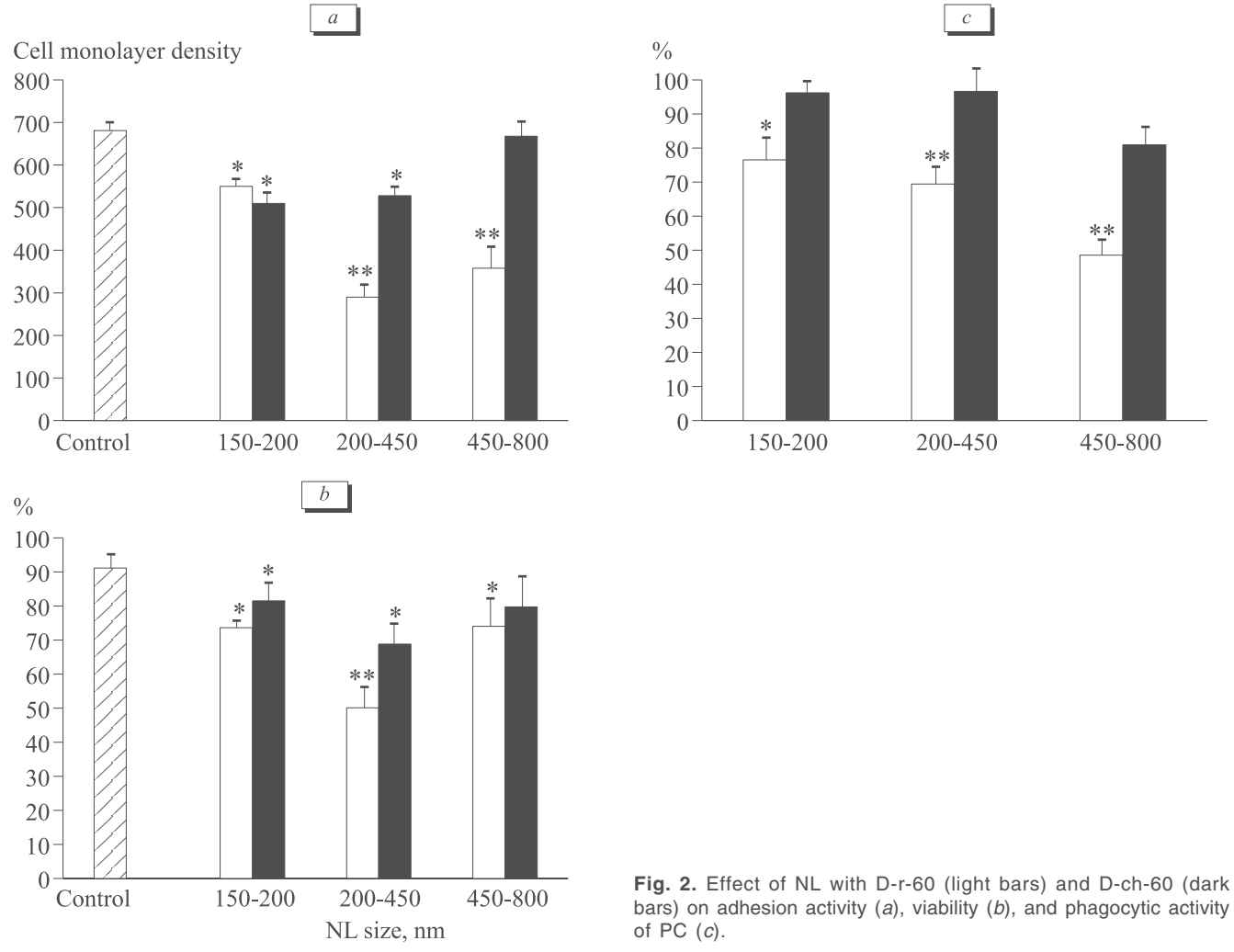

Fig. 2. Effect of NL with D-r-60 (light bars) and D-ch-60 (dark bars) on adhesion activity (*a*), viability (*b*), and phagocytic activity of PC (*c*).

after addition of D-r-35- and D-r-60-containing NL. The number of phagocytic macrophages was minimum after treatment with D-r-60-containing NL of 450-800 nm. It was probably associated with lower stability of these NL and rapid elimination of D-r-60 (Fig. 2). The cytotoxic effect of OD_r-containing NL did not depend on the molecular weight of dextran.

We conclude that the biocompatibility of OD_c-containing NL is higher than that of OD_r-containing NL. They hold much promise as a biocompatible container for targeted transport of bioactive substances and drugs into the target cells and tissues.

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