Phagocytic Activity of Macrophages against Liposomes with Conjugates of Oxidized Dextrans and Isonicotinic Acid Hydrazide during Modeling of Phagocytosis Disturbances *In Vitro*

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We studied phagocytic activity of macrophages against molecular-liposome hybrid compositions consisting of liposomes (diameter 200-450 nm) containing oxidized dextrans with a molecular weight of 35 or 60 kDa conjugated with the basic antituberculosis preparation isonicotinic acid hydrazide (dextrazides) during modeling of various disturbances of endocytosis function of phagocytic cells *in vitro*. Preincubation of macrophages with trypsin, colchicine, or sodium azide did not change the parameters of adhesion of molecular-liposome hybrid compositions to macrophages. It was found that preincubation of cells with colchicine or sodium azide reduced parameters of phagocytosis of the molecular-liposome hybrid compositions; this reduction did not depend on the molecular weight of dextrans entering the composition of the molecular-liposome hybrid compositions.

Key Words: *oxidized dextrans; isonicotinic acid hydrazide; liposomes; liposomes; phagocytosis; macrophages*

Tuberculous inflammation is characterized by the formation of granulomas consisting of macrophages with persisting *M. tuberculosis.* This determines the need in targeted delivery of drugs (better, of corpuscular nature) to macrophages for capture via phagocytosis, the most effective route of endocytosis for these cells; this allows reducing the load to hepatocytes (phagocytosis is not characteristic of these cells).

We previously showed that molecular-liposomal hybrid compositions (MLHC) containing oxidized dextrans (OD) conjugated with isonicotinic acid hydrazide can be considered as a promising vehicle for targeted delivery of the antituberculosis drug to cells of the mononuclear phagocyte system [3]. However, the mechanisms limiting phagocytosis of MLHC by macrophages are little studied.

Here we studied mechanisms of phagocytosis of MLHC consisting of liposomes containing OD with a molecular weight of 35 and 60 kDa conjugated with isonicotinic acid hydrazide (INAH) and factors potentially limiting MLHC capture under conditions of experimental endocytosis disturbances *in vitro*.

MATERIALS AND METHODS

In vitro experiments were carried out on peritoneal exudate cells from BALB/c mice (2-month-old males

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weighing 21-22 g, obtained from Nursery of Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk). Peritoneal cells (PC) were isolated after animal sacrifice via routine cervical dislocation (under ether anesthesia) [1]. Methods of dextran oxidation and conjugation with INAH and preparation of MLHC were described previously [2-4]. PC were cultured on coverslips (10^6 cells in 2 ml medium 199 containing 10% embryonic calf serum) at 37°C. The cells were treated with trypsin (0.25% solution; 10 min at 37°C) for modeling damage to the membrane receptor apparatus [5], with colchicine (final dilution 5 μg/ml, at 30 min at 25°C) for modeling damages to cell cytoskeleton [6], with sodium azide (final dilution 0.5 mg/ml, at 30 min at 25°C) for inhibition of oxidative metabolic process in cell membranes, including those regulating migration of surface cluster differentiation molecules and their capping [7].

Adhesion properties of MLHC were evaluated by their adhesion to macrophages (MP). MLHC (diameter 200-450 nm) including dextrazide-35 or dextrazide-60 were added to PC during the 3rd hour in culture. Adhesion to MP plasmalemma and phagocytic activity were evaluated 5 min and 24 h after addition of MLHC to the cell cultures. After incubation with MLHC, the cell cultures were fixed in 4% formaldehyde (in phosphate buffer, 10 min). For detection of MLHC on the surface and in MP, the cell culture preparations were stained with lipophilic dye sudan black B (0.25% solution in 70% ethanol). For evaluation of MLHC number on MP, the cell culture preparations were placed on a slide with cell-coated side up, and during evaluation of phagocytosis with cell-coated side down. The cells were photographed with an AxioCamHr camera using an AxioImager Z1 microscope (×100, Zeiss), motorized module for optical scanning, and AxioVision 4.6 software (Zeiss). For evaluation of adhesion capacity of MLHC we used photos of two upper optical sections of cells (200 nm step) showing MLHC adherent to MP. The percent of MP with adherent MLHC and an arbitrary parameter of MLHC quantity adherent to one target cell (MP) were calculated. For evaluation of MLHC phagocytosis by MP, the total number of MP and the number of MP containing stained MLHC in the cytoplasm were determined. The phagocytic index (PI) was calculated by the formula:

$$PI=(N_p\ MP/N_t\ MP)\times 100\%,$$

where N_p is the number of MP phagocytizing MLHC and N_t is the total number of MP. The index of MLHC adhesion capacity and arbitrary parameter of the amount of MLHC phagocytized by MP were evaluated by computer Morphometry using Video-Test-Morpho 3.2 software. Digital cell images were binarized by sudan black color and the total area of all binary images of MLHC was taken as an arbitrary parameter of their total content on MP surface or arbitrary parameter of MLHC quantity phagocytized by MP. Significance of differences between the mean values of the studied parameters for experimental cultures was evaluated using nonparametric White test. The data are presented as $M\pm m$, the differences were significant at $p<0.05$. The data were processed using Statistica 8 software.

RESULTS

Pretreatment with trypsin did not inhibit adhesion of MLHC to MP (Fig. 1). Incubation with colchicine also did not affect the adhesion properties of MP. Sodium azide slightly weakened MLHC adhesion to MP. Thus, enzymatic elimination of protein molecules with receptor functions from the phagocyte surface does not play a critical role in adhesion of MLHC to MP membranes. A minor decrease in phagocytic properties after short-term treatment with sodium azide suggests that deceleration of metabolic processes in cell membranes and probably their depolarization can weaken adhesion activity of phagocytes against MLHC with encapsulated dextrazides.

Treatment with trypsin had no effect on PI of MP during evaluation of MLHC phagocytosis (Fig. 2). In contrast, incubation with colchicine considerably decreased this parameter in all experimental groups irrespective of the molecular weight of oxidized dextrans entering the composition of MLHC. A more pronounced decrease in PI of MP was noted after cell pretreatment with sodium azide.

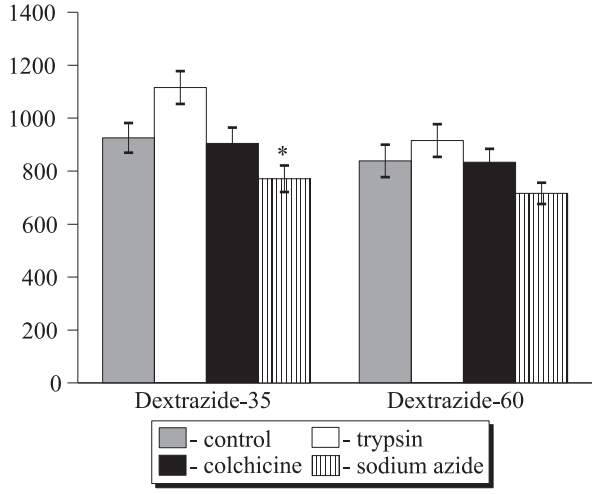

Fig. 1. Adhesion activity of MP after incubation with trypsin, colchicine, and sodium azide against MLHC containing dextrazide-35 and dextrazide-60 (by index of adhesion activity reflecting the amount of MLHC adherent to MP). *$p<0.05$, **$p<0.01$ compared to the control (without pretreatment).

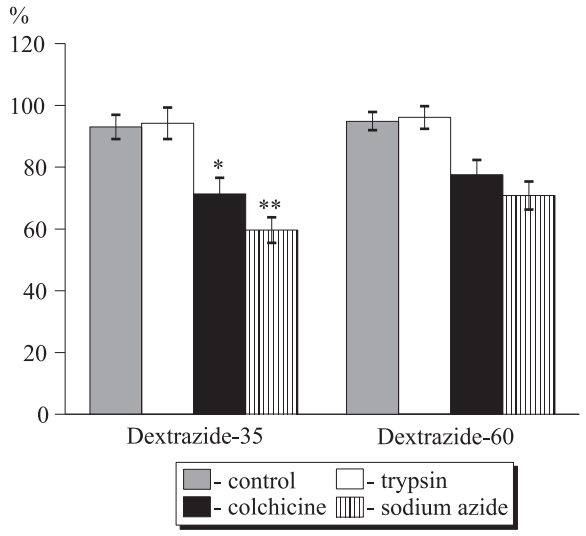

Fig. 2. Phagocytic activity of peritoneal MP after incubation with trypsin, colchicine, and sodium azide against MLHC containing dextrazide-35 and dextrazide-60 (by the number of MP phagocytizing MLHC, %) after their addition to cultured peritoneal cells. *p<0.05, **p<0.01 compared to the control.

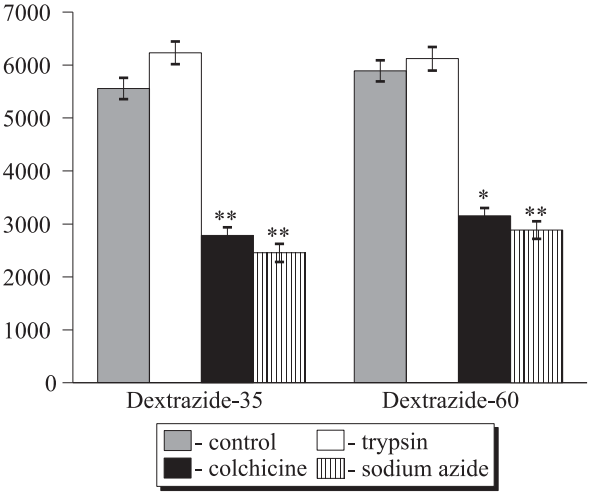

Fig. 3. Phagocytic activity of MP in culture of peritoneal cells after incubation with trypsin, colchicine, and sodium azide against MLHC containing dextrazide-35 and dextrazide-60 (by arbitrary index of phagocytic activity reflecting the amount of MLHC phagocytized by MP) after their addition to cultured peritoneal cells. Ordinate: amount of MLHC captured by MP. *p<0.05, **p<0.01 compared to the control.

Treatment with trypsin had no effect on another important parameter of phagocytic activity of MP against MLHC, the relative amount of MLHC phagocytized by MP (Fig. 3). Incubation with colchicine or sodium azide significantly reduced this parameter in all experimental groups (by 50% on average, compared to the control) irrespective on the molecular weight of OD.

Thus, phagocytosis of MLHC containing OD conjugated with INAH is determined by the intensity of metabolic processes in phagocyte plasmalemma contacting with phagocytized particles and by mobility of cell cytoskeleton responsible for effective invagination of cell membranes during phagocytosis. Damage to the receptor apparatus of mononuclear phagocytes, reduced the level of metabolism in their membranes, and disturbances in cell cytoskeleton did not appreciably impair adhesion activity of MP against MLHC containing OD conjugated with INAH. This is determined by unspecific binding of MLHC containing OD conjugated with INAH with cell surface, which depends on physicochemical properties of MLHC (lipophilic properties of membrane, surface charge) and on properties of cell membranes (probably by affinity of lipid components of cell membranes and MLHC membranes, differences in electric potential of MLHC membranes and cell membranes.

These findings suggest that dextrazides encapsulated in liposomes can be effectively captured by macrophages thus providing targeted delivery of INAH to *M. tuberculosis* persisting in the vacuolar apparatus of macrophages.

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