NANOTECHNOLOGY

In Vitro Effects of Molecular Nanosomal Hybrid Compositions with Oxidized Dextrans, Conjugated with Isonicotinic Acid Hydrazine on Peritoneal Macrophages

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The effects of molecular nanosomal hybrid compositions consisting of nanoliposomes with oxidized dextrans (mol. weights 35 and 60 kDa) conjugated with isonicotinic acid hydrazine (dextrazides) on peritoneal macrophages were studied *in vitro*. Incubation of peritoneal cells with molecular nanosomal hybrid compositions modified the immunological phenotype of macrophage populations, which reflected an increase in their functional activity. Molecular nanosomal hybrid compositions containing dextrazide with 60-kDa dextran more effectively activated macrophages.

Key Words: *oxidized dextranes; isonicotinic acid hydrazide; nanoliposomes; peritoneal macrophages*

One of important characteristics of agents designed for target delivery of bioactive substances and modulation of the biological target (including the substances based on endocytic activity of cells) is the presence of intrinsic bioactive properties potentiating the efficiency of the drugs delivered to the biological targets [3]. We previously showed that nanoliposomes containing oxidized dextrans obtained by the chemical (permanganate) method are highly biocompatible, tropic for mononuclear phagocytes, and can be regarded as prospective biocompatible containers for bioactive substances and drugs for their targeted delivery to macrophages and target organs [4]. However, biological characteristics of these compositions and their possible effects on functional activity of macrophages are little studied.

We studied the effects of molecular nanosomal hybrid compositions (MNHC) containing dextrans conjugated with isonicotinic acid hydrazide (dextrazides) with different molecular weights of dextran matrices on the peritoneal macrophages.

MATERIALS AND METHODS

Experiments were carried out *in vitro* on peritoneal transudation cells from BALB/c mice (2-month-old females weighing 21-22 g, Breeding Center of Institute of Cytology and Genetics). Peritoneal cells (PC) were isolated after sacrifice by cervical dislocation under ether narcosis [1]. The possible bio-

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active characteristics of MNHC were evaluated by the immunocytochemical method 24 h after addition of MNHC to the primary cultures of PC during the third hour after their inoculation in culture. The following MNHC were studied: MNHC including dextrazide-ch-35 (chemically oxidized dextran with a molecular weight of 35 kDa conjugated with isonicotinic acid hydrazine) and dextrazide-ch-60 (chemically oxidized dextran with a molecular weight of 60 kDa conjugated with isonicotinic acid hydrazine), both MNHC in the same nanosize range for nanoliposomes (200-450 nm). Methods for dextran oxidation, their conjugation with isonicotinic acid hydrazine, and derivation of nanoliposomal MNHC on this base are described previously [2,4,5]. Peritoneal cells were cultured on slides (10^6 cells in 2 ml medium 199 with 10% FCS) in glass flasks at 37°C. Macrophages (MP) expressing CD25 differentiation cluster were detected in PC cultures by indirect immunocytochemical staining using monoclonal antibodies to CD25 (Biotin Rat Anti-Mouse CD25; Isotype: Rat IgM; Clone: 7D4). Standard Novostain 500 kit (BD) served as the visualization system.

The percentage of peritoneal MP expressing several differentiation clusters simultaneously (CD14, CD16/32, and CD25) was evaluated by flow cytofluorometry on a FACSCalibur two-laser flow cytofluorometer. Macrophages with the following phenotypes were evaluated: $CD14^-CD25^-$, $CD14^-CD25^+$, $CD14^+CD25^-$, $CD14^+CD25^+$, $CD16/32^-CD25^-$, $CD16/32^-CD25^+$, $CD16/32^+CD25^-$, and $CD16/32^+CD25^+$. Possible bioactive characteristics of MNHC were evaluated 24 h after addition of the studied MNHC into primary PC cultures (in culturing tubes in a final dilution of 1:100). The suspension of PC (0.5×10^6 cells in 0.5 ml RPMI-1640 with glutamine and 10% FCS) were cultured at 37°C for 24 h in the Eppendorf plastic tubes. The subpopulation composition of peritoneal MP was studied on a flow cytofluorometer in a fluid for flow cytometry (BD FACS_Flow). The following monoclonal antibodies served as typing antibodies: PE-anti-mouse CD14 (rm C5-3, Cat. No. 553740), PE-anti-mouse CD16/CD32 (Fcg III/II Receptor/(2.462), Cat. No. 553145), APC rat anti-mouse CD25 (IL-2 Receptor a chain, p55, Cat. No. 557192) (Becton Dickinson). A total of 3500-5000 viable cells getting into a respective gate and discriminated by the fluorescence intensity were analyzed for each sample. The data of forward and side light scatter of the studied cells were shown during the study at two-dimensional histograms (FSC/SSC). The gate corresponding to macrophage cell population was selected by the proportion of FSC and SSC parameters; the presence of cells with CD14 phenotype in the gate served as specific control of isolation of the analyzed MP subpopulation.

The significance of differences between the studied experimental groups of cultures was evaluated using nonparametric White test. The data are presented as $M\pm m$, the differences were considered significant at $p<0.05$. The data were statistically processed using Statistica 5.0 software.

RESULTS

Preliminary evaluation of the effect of MNHC on functional activity of MP was carried out by the immunocytochemical method, *i.e.* by changes in the expression of CD25 differentiation cluster (one of MP activation markers) on MP membranes. Addition of MNHC (in a final dilution of 1:200) to MP-containing PC culture led to an increase in the percentage of CD25-expressing MP from 0.31±0.03% (control) to 0.55±0.04% (MNHC containing dextrazide-ch-35) and to 1.80±0.06% (MNHC containing dextrazide-ch-60; $p<0.01$).

Flow cytofluorometry was used for more precise evaluation of the effect MNHC on changes in functional activity of MP associated with their immunological phenotype. Macrophages were stimulated with MNHC in a final dilution of 1:100.

The effects of MNHC (in a 200-450 nm nanosize range) with dextrazide-ch-35 and dextrazide-ch-60 on the expression of CD14, CD16/32, and CD25 receptor markers on peritoneal MP studied by flow cytofluorometry are summed up in Figs. 1 and 2.

In the control (addition of buffer solution to PC cultures), the majority (about 85%) of MP did not express CD14 and CD25 markers and were referred to $CD14^-CD25^-$ MP (Fig. 1). Incubation of PC with MNHC containing different dextrazides reduced the $CD14^-CD25^-$ pool. The content of $CD14^-CD25^+$ MP decreased in cultures to which nanoliposomes containing dextrazide-ch-35 were added. The subpopulation of MP expressing CD14 and CD25 markers ($CD14^+CD25^+$ MP subpopulation) increased significantly in these cultures. Hence, the total content of MP expressing CD25 also significantly increased in comparison with the control (by more than 1.5 times). This effect was most pronounced for cultures in which functional activity of MP was modulated by nanoliposomes containing dextrazide-ch-60. The percentage of $CD14^+CCD25^+$ MP increased more than 3-fold in comparison with the control after MP incubation with nanoliposomes containing dextrazide-ch-60.

In the control, only 35% MP did not express CD16/CD32 and CD25 markers and were referred

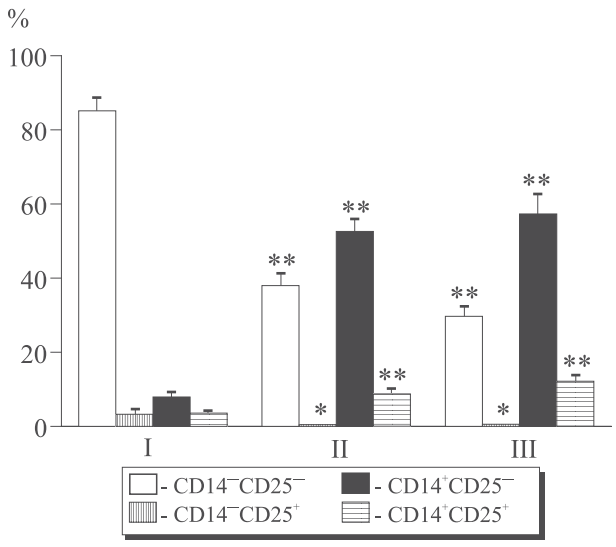

Fig. 1. Effect of MNHC (200-450 nm) with dextrazide-ch-35 and dextrazide-ch-60 on expression of CD14 and CD25 markers on MP (shown by flow cytofluorometry) 24 h after addition of MNHC into PC culture. Light bars: row 1 ($CD14^-CD25^-$); vertically-hatched bars: row 2 ($CD14^-CD25^+$); dark bars: row 3 ($CD14^+CD25^-$); horizontally-hatched bars: row 4 ($CD14^+CD25^+$). Ordinate: pool of MP expressing CD14 and CD25 markers. Here and in Fig. 2: I) common control (intact cultures); II) dextrazide-ch-35; III) dextrazide-ch-60. *p<0.05, **p<0.01 compared to the control (addition of buffer solution).

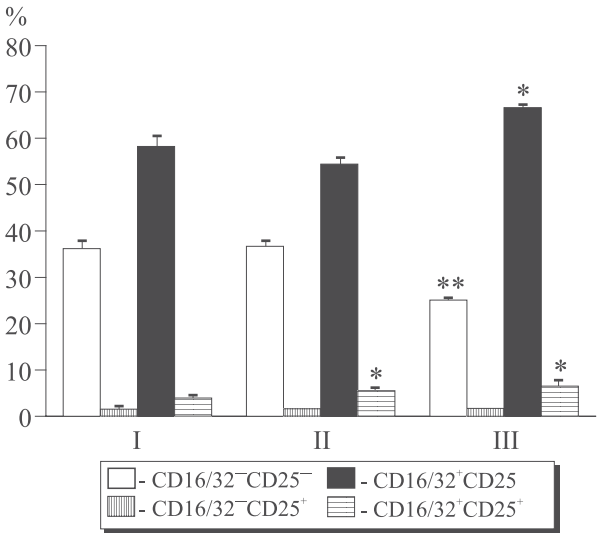

Fig. 2. Effect of MNHC (200-450 nm) with dextrazide-ch-35 and dextrazide-ch-60 on expression of CD16/32 and CD25 markers on MP (shown by flow cytofluorometry) 24 h after addition of MNHC into PC culture. Light bars: row 1 ($CD16/32^-CD25^-$); vertically-hatched bars: row 2 ($CD16/32^-CD25^+$); dark bars: row 3 ($CD16/32^+CD25^-$); horizontally hatched bars: row 4 ($CD16/32^+CD25^+$). Ordinate: pool of MP expressing CD16/32 and CD25 markers.

to the population of $CD14^-CD25^-$ MP (Fig. 2). About 60% of these MP were referred to the subpopulation of $CD16/32^+CD25^-$ MP. A similar trend was detected for MNHC specimens containing dextrazide-ch-35. On the other hand, the total percentage of MP expressing the CD16/CD32 marker virtually did not change, because the pool of $CD16/32^+CD25^+$ MP expressing CD16/CD32 and CD25 differentiation clusters on their membranes simultaneously increased significantly in these experimental cultures. The pool of $CD16/32^+CD25^+$ MP increased similarly in PC cultures incubated with dextrazide-ch-60-containing MNHC. It was found that MNHC containing dextrazide-ch-60 significantly increased the expression of total polymorphic epitope on extracellular domains of the cell — CD16 (Fcg III receptor) and CD32 (Fcg II receptor).

Hence, all the studied MNHC samples containing dextrazide-ch-35 and dextrazide-ch-60 (of the same nanosize range of 200-450 nm) activated phagocytic macrophagal cells. Macrophage activation was more pronounced under the effect of MNHC containing dextrazide with of 60-kDa dextran.

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