Effects of Molecular Liposomal Hybrid Compositions with Oxidized Dextrans and Isonicotinic Acid Hydrazide on Production of Granulocytic Macrophage Colony-Stimulating Factor by Macrophages

V. A. Shkurupy, S. A. Arkhipov, A. V. Troitsky, N. G. Luzgina, M. V. Zaikovskaja, E. G. Ufimceva, D. A. Iljine, E. S. Akhramenko, E. P. Gulyaeva, and T. N. Bistrova

Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 4, pp. 219-221, December, 2009
Original article submitted September 29, 2009

The effects of molecular liposomal hybrid compositions consisting of liposomes (200-450 nm) containing oxidized dextrans (dextranals; 35-60 kDa) conjugated with isonicotinic acid hydrazide (dextrazides), their components, and native dextrans on the production of granulocytic macrophage CSF by peritoneal macrophages were studied *in vitro*. Dextranals proved to be more potent inductors of granulocytic macrophage CSF than native dextrans. Conjugation of nicotinic acid hydrazide with dextranals did not modify their capacity to stimulate the production of granulocytic macrophage CSF. Liposomes in the molecular liposomal hybrid compositions did not attenuate the dextrazide capacity to stimulate the production of granulocytic macrophage CSF. Molecular liposomal compositions containing 60 kDa dextrazide exhibited the most potent stimulatory effect on macrophage production of granulocytic macrophage CSF.

Key Words: *oxidized dextrans; isonicotinic acid hydrazide; liposomes; macrophages; granulocytic macrophage colony stimulating factor*

Study of the drug characteristics, specifically, biocompatibility, biotropism, biological activity, and possibility of delivery to the target cells is an obligatory part of drug creation [2]. It was shown that liposomes containing oxidized dextrans (OD) obtained by chemical oxidation are characterized by high biocompatibility, are tropic for the mononuclear phagocyte system (MPS) cells, and can be regarded as prospective biocompatible containers for bioactive substances and drugs for their addressed delivery to the target organ macrophages [4], for example, isonicotinic acid hydrazide (INAH), a drug for therapy of tuberculosis. It was found that these molecular liposomal hybrid compositions (MLHC) consisting of liposomes containing OD (with mol. weights of 35 or 60 kDa) conjugated with INAH (dextrazides), incubated *in vitro* with peritoneal macrophages, modulated the immunological phenotype of macrophages and stimulated their functional activity [3].

We studied the possible effects of dextran oxidation, their conjugation with INAH, and their packing in liposomes on the production of granulocytic macrophage CSF (GM-CSF), one of the main regulators of systemic MPS cell response, by macrophages.

MATERIALS AND METHODS

Experiments were carried out *in vitro* on peritoneal transudate cells from BALB/c mice (2-month-old

Center of Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** sck@soramn.ru. V. A. Shkurupy

0007-4888/09/1484-0710

males, 21-22 g from Breeding Center of Institute of Cytology and Genetics, Novosibirsk). Peritoneal cells were obtained after sacrifice by routine cervical dislocation under ether narcosis [1]. The production of GM-CSF by macrophages was evaluated by immunocytochemical method 24 h after addition of dextran with a molecular weight of 35-40 kDa (final dilution 125 μg/ml) and 60-75 kDa (125 μg/ml); dextranal, 35-40 kDa (125 μg/ml) and 60 kDa (125 μg/ml); dextrazide, 35-40 kDa (125 μg/ml) and 60 kDa (125 μg/ml); INAH (5 μg/ml); "empty" liposomes with Tris buffer, and liposomes with INAH (5 μg/ml in the final dilution of liposomes), dextrazide, 35-40 kDa (125 μg/ml) or 60 kDa (125 μg/ml) — MLHC. Liposomes and MLHC of similar size (200-450 nm) were used in the experiments. The concentration of liposomes in medium for culturing was equal (in the final dilution) in all experimental groups of cultures. Dextran oxidation, conjugation with INAH, preparation of liposomal MLHC forms on this base, and their standardization were carried out as described previously [3-6]. Peritoneal cells were cultured on slides (10^6 cells in 2 ml medium 199 with 10% fetal calf serum) in glass flasks at 37°C. The cells were fixed in 4% formaldehyde (in phosphate buffer). Antigenic determinants of GM-CSF were demasked by incubation with Triton X-100 (0.3% solution in phosphate buffer). Cell cultures were incubated for 1 h with primary antibodies to GM-CSF (Rat Anti-Mouse GM-CSF; Isotype: Rat IgG2a), with biotin-conjugated goat anti-rat Ig specific polyclonal antibody in a humid chamber (30 min), and with streptavidine–peroxidase complex (30 min). The preparations were stained with diaminobenzidene (DAB) solution with H_2O_2-containing substrate (standard BD DAB kit). For cell visualization, the preparations were post-stained with 1% methylene green solution. Cell membrane or cytoplasm zones containing GM-CSF were specifically stained dark-brown. The intensity of staining was directly proportional to the amount of the factor produced. The expression of GM-CSF in the population of phagocytic cells was evaluated by the percentage of cells in which the factor was detected. The levels of GM-CSF production in macrophages was also scored as follows: 1 point corresponded to low production (less than 1/3 cytoplasm specifically stained with DAB), 2 points indicated mean production (1/2-2/3 cytoplasm stained with DAB), and 3 points indicated high production (more than 2/3 of the cytoplasm stained with DAB). The mean value of this parameter was calculated for each experimental group of cultures.

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

RESULTS

Twenty-four hours after addition of native oxidized dextrans (mol. weights 35-40 and 60-75 kDa) to PC cultures, the number of GM-CSF-producing macrophages increased more than 2-fold in comparison with the control (Fig. 1). The stimulatory effect of OD on GM-CSF production was more pronounced. The percentage of macrophages producing GM-CSF reached 42% of all macrophages in culture after incubation with dextranal-35 and 55% with dextranal-60. Conjugation of INAH with OD (dextrazides) did not modify the capacity of dextranals in this complex to stimulate the production of GM-CSF: the percentage of GM-CSF-producing macrophages incubated with dextrazide-35 and dextrazide-60 reached 47 and 61%, respectively, of the total number of studied macrophages. Incubation of PC with INAH showed a trend to a reduction of the percentage of GM-CSF-producing cells, presumably because of inhibition of this process with free INAH, while INAH packed in liposomes exhibited no inhibitory effect of this kind, presumably due to liposome shielding of INAH during the exposure period used in the experiment. Liposomes containing buffer solution did not stimulate GM-CSF production by macrophages. Liposomes in MLHC did not attenuate the dextrazide capacity to stimulate the production of GM-CSF in macrophages. The MLHC containing dextrazide with mol. weight of 60 kDa ex-

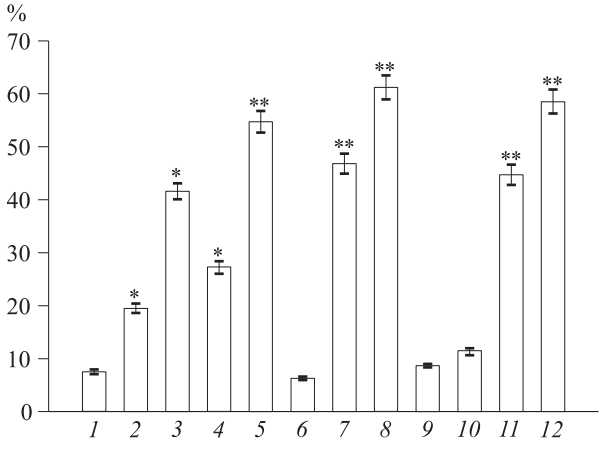

Fig. 1. Effects of MLHC, their components (INAH, dextrazides, liposomes), native and oxidized dextrans on the number of GM-CSF-producing macrophages 24 h after their addition into peritoneal cell cultures. Here and in Fig. 2: 1) control (intact cultures); 2) dextran, 35-40 kDa; 3) dextran, 60-75 kDa; 4) dextranal, 35 kDa; 5) dextrazide, 35 kDa; 6) dextranal, 60 kDa; 7) dextrazide, 60 kDa; 8) INAH; 9) empty liposomes with Tris buffer; 10) liposomes with INAH; 11) liposomes with dextrazide, 35 kDa (MLHC); 12) liposomes with dextrazide, 60 kDa (MLHC). *$p<0.05$, **$p<0.01$ compared to the control.

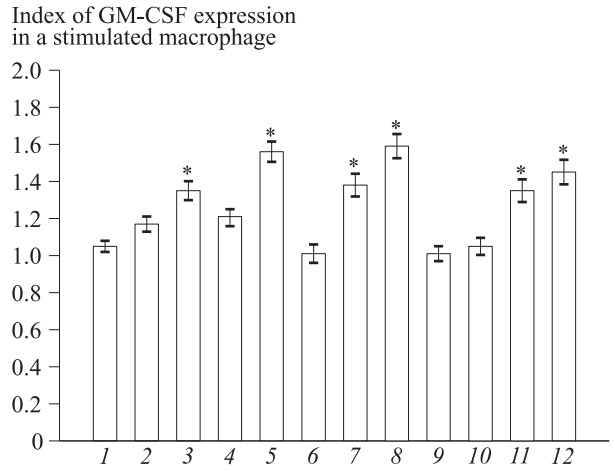

Fig. 2. Effects of MLHC, their components (INAH, dextrazides, liposomes), native and oxidized dextrans on the levels of GM-CSF production by macrophages by arbitrary index of expression 24 h after their addition to peritoneal cell cultures.

hibited the highest stimulatory activity on GM-CSF production by macrophages. Incubation of PC with MLHC containing dextrazide with mol. weight of 60 kDa led to a 6-fold increase in the percentage of GM-CSF-producing macrophages in comparison with the intact control (without any additional substances in standard culture medium).

Study of the effects of MLHC and other components of MLHC on the levels of GM-CSF production by individual macrophages (Fig. 2) showed regularities of GM-CSF production induction similar to those detected in evaluation of a pool of GM-CSF-producing macrophages (Fig. 1). Evaluation of MLHC effect on the levels of GM-CSF expression in individual macrophages showed an approximately 30% increase in the value reflecting the volume of produced factor in response to addition of liposomes with dextrazides-35 and dextrazide-60 to the culture medium (Fig. 2). The maximum stimulation of GM-CSF production (45%) was observed in response to MLHC with dextrazide-60.

Hence, MLHC containing dextrazides can be regarded as compositions with high immunostimulatory and immunomodulatory potentials mediated by macrophages through GM-CSF production. Dextran oxidation led to not only an increase of their own biological activity and capacity to stimulate the MPS cells; they could be conjugated with another bioactive substance (with INAH in this study) retaining the acquired characteristics in the conjugate with OD and in MLHC.

The study was carried out within the framework of Federal Target Program “Studies and Developments in Priority Trends of Research and Technological Complex of Russia for 2007-2012” (State Contract No. 02.513.11.3183 of April 23, 2007).

REFERENCES

1. E. D. Goldberg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture Methods in Hematology* [in Russian], Tomsk (1992).
2. V. A. Shkurupy, *Tuberculous Granulomatosis, Cytophysiology and Address Therapy* [in Russian], Moscow (2007).
3. V. A. Shkurupy, S. A. Arkhipov, V. O. Tkachev, *et al.*, *Byull. Eksp. Biol. Med.*, **146**, No. 11, 563-566 (2008).
4. V. A. Shkurupy, S. A. Arkhipov, A. V. Troitsky, *et al.*, *Ibid*, **145**, No. 1, 123-126 (2008).
5. V. A. Shkurupy, S. A. Arkhipov, A. V. Troitsky, *et al.*, *Ibid*, **145**, No. 1, 120-122 (2008).
6. V. A. Shkurupy, Yu. N. Kurunov, O. V. Grishin, *et al.*, *A Method for Obtaining Long-Acting Isoniazide*, Patent RU No. 2143900, 10.01.2000.