Phagocytosis of Hybrid Molecular Nanosomal Compositions Containing Oxidized Dextrans Conjugated with Isonicotinic Acid Hydrazide 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 *Byulleten' Experimental'noi Biologii i Meditsiny*, Vol. 148, No. 12, pp. 633-635, December, 2009
Original article submitted April 23, 2009

We studied phagocytic activity of macrophages towards hybrid molecular nanosomal compositions consisting of 150-800-nm nanoliposomes containing oxidized dextrans with a molecular weight of 35 and 60 kDa obtained by chemical ("permanganate") and radiochemical oxidation of dextran conjugated with isonicotinic acid hydrazide (dextrazides, intracellular prolonged antituberculous drugs). Phagocytic activity of macrophages towards hybrid molecular nanosomal compositions containing dextrazides obtained by chemical oxidation of dextrans is higher than activity towards hybrid molecular nanosomal compositions containing dextrazides prepared by radiochemical oxidation and depends on the size of hybrid molecular nanosomal compositions and molecular weight of oxidized dextrans.

Key Words: *chemically and radiochemically oxidized dextrans; isonicotinic acid hydrazide; nanoliposomes; peritoneal macrophages; phagocytosis*

Endocytosis can provide an efficient strategy for delivery of compositions with predetermined pharmacokinetic properties into cells by using various types of vehicles (from molecular to nano- and microcarriers) [4,6]. This will enhance specificity and efficacy of bioactive substances (BAS) delivered to target cells and therefore will reduce their concentration in the organism and the risk of posttoxic complications. It was previously demonstrated that conjugates of isonicotinic acid hydrazide (INAH) with oxidized dextran are lysosomotropic [3,6], while hybrid molecular nanosomal compositions (HMNC) prepared from dextrans oxidized by chemical ("permanganate") technique are characterized by higher biocompatibility and tropism towards macrophages than HMNC prepared from dextran oxidized using radiochemical technique [7]. The complex of oxidized dextran (OD) with HMNC demonstrated high antimycobacterial activity under experimental conditions [3]. OD solutions prepared using radiochemical technique can contain various peroxide admixtures [2,8] modulating OD biocompatibility. From a practical perspective, HMNC containing OD conjugated with BAS (*e.g.* with INAH, a basic antituberculosis drug), are of particular interest. In this context, it is important to compare various HMNC in *in vitro* tests, including tests characterizing their tropism to phagocytes, the site of persistence of tuberculosis mycobacteria.

The aim of the study was to investigate specific features of phagocytosis of HMNC containing chemically and radiocmemically oxidized dextrans conjugated with INAH by macrophages depending on the size of nanoliposomes and molecular weight of OD.

Research 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

MATERIALS AND METHODS

In vitro experiments were performed on macrophages isolated from peritoneal transudate of BALB/c mice (2-month-old males weighing 21-22 g; nursery of Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Medical Sciences). The objects of investigation were macrophages and nanoliposomal forms of HMNC of different size (150-200 nm, 200-450 nm and 450-800 nm) including the following dextrazides: dextrazide-ch-35 (chemically oxidized 35 kDa dextran, conjugated with INAH); dextrazide-ch-60 (chemically oxidized 60 kDa dextran, conjugated with INAH); dextrazide-r-35 (radiochemically oxidized 35 kDa dextran, conjugated with INAH), and dextrazide-r-60 (radiochemically oxidized 60 kDa dextran, conjugated with INAH). Methods of dextran oxidation (their advantages and disadvantages), conjugation with INAH, and preparation of nanoliposomal HMNC forms were described previously [4,5,7]. The animals were sacrificed by cervical dislocation under ether anesthesia and peritoneal cells were isolated routinely [1], cultured on coverslips (10^6 cells per 2 ml medium 199 containing 10% embryonic calf serum) in glass vials at 37°C [3]. Phagocytic activity of macrophages, components of peritoneal cell culture, was evaluated 24 h after addition of HMNC to the culture medium. After incubation with HMNC, the cell cultures were fixed with 4% formaldehyde (4% aqueous solution in phosphate buffer). For HMNC detection, the cells were stained with lipophylic dye Sudan black B (0.25% solution in 70% ethanol), washed in distilled water, and stained with safranin (1% aqueous solution). Stained specimens were put on slides with cells "looking downwards". Phosphate buffer solution was introduced between the slide and cover glass. The cells were photographed using an AxioI-mager Z1 microscope (Zeiss) using AxioCamHr digital camera at fixed objective magnification 40. The total numbers of macrophages and macrophages containing stained HMNC in the cytoplasm were determined in 10 fields of view at ×40. Phagocytosis index was calculated as the ratio of the number of macrophages with phagocytized HMNC to the total number of macrophages multiplied by 100%.

The arbitrary parameter, number of phagocyted HMNC was estimated by computer morphometry using VideoTest-Morpho 3.2 software. Digital images of cells (×100) were binarized by the color of Sudan black dye and the sum of squares (pixel^2) of all binary images of HMNC stained with Sudan Black was used as relative index of total HMNC content per one macrophage (10 fields of view were analyzed). Significance of difference between the compared mean values of the studied parameters in the experimental cultures was estimated 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 5.0 software.

RESULTS

Accumulation of HMNC in macrophages varied depending on HMNC size, method of dextran oxidation, and their molecular weight. (Figs. 1 and 2). The percent of macrophages phagocyting HMNC tended to decrease for HMNC containing radiochemically oxidized 150-450-nm dextrans. For 450-800-nm HMNC, this parameter was considerably higher for HMNC containing chemically oxidized dextrans. HMNC capture by macrophages tended to decrease with increasing the size of nanoliposomes entering the HMNC.

These regularities were most clearly seen during estimation of arbitrary parameter of HMNC number captured by one macrophage. For all HMNC size ranges, phagocytosis indices were higher for HMNC containing dextrazides synthesized on the basis of chemically oxidized dextrans, irrespective of the molecular weight of OD used for dextrazide preparation. The number of captured HMNC decreased with increasing their size. Higher values of phagocytosis were noted for HMNC containing 35 kDa OD compared to HMNC containing 60 kDa OD, irrespective of the oxidation method.

Thus, the most efficient capture by macrophages was observed for 150-200-nm HMNC and 200-450-nm

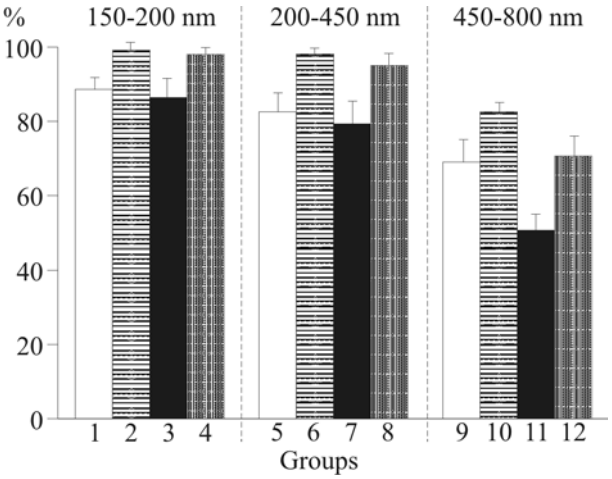

Fig. 1. Results of estimation of *in vitro* phagocytic activity of peritoneal macrophage towards HMNC depending on HMNC size (nm), molecular weight (kDa) of chemically (-ch-) and radiochemically (-r-) oxidized dextrans conjugated with INAH by the percentage of macrophages phagocytizing MNHC. Each experimental group consisted of 5 cultures. Here and on Fig. 2: light bars: MNHC-OD-r-35 kDa, horizontal hatching: MNHC-OD-ch-35 kDa, dark bars: MNHC-OD-r-60 kDa, vertical hatching: MNHC-OD-ch-60 kDa. $p<0.05$ between groups 11 и 12; $p<0.01$ between groups 1 and 9, groups 3 and 11, and groups 4 and 12.

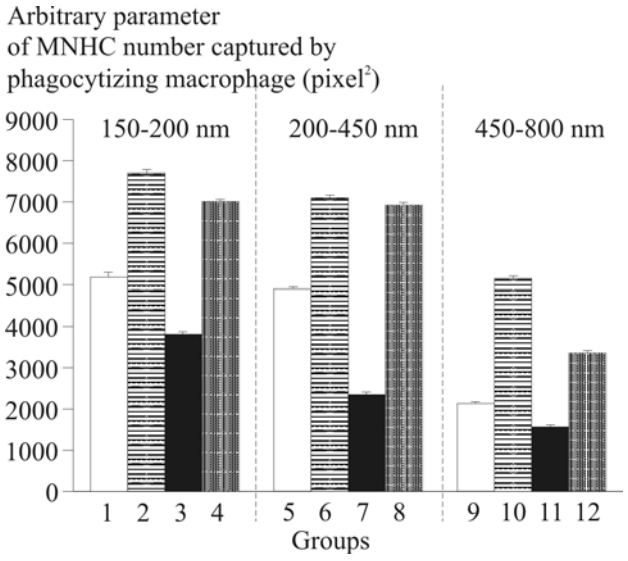

Fig. 2. Results of estimation of *in vitro* phagocytic activity of per 3oneal macrophage towards HMNC depending on HMNC size (nm), molecular weight (kDa) of chemically (-ch-) and radiochemically (-r-) oxidized dextrans conjugated with INAH by arbitrary parameter of MNHC number captured by phagocytizing macrophages (pixel²). $p<0.05$ between groups 5 and 9, groups 1 and 3, and groups 10 and 12; $p<0.01$ between groups 1 and 2, groups 3 and 4, groups 5 and 6, groups 7 and 8, groups 9 and 10, groups 11 and 12; groups 1 and 9, groups 2 and 10, groups 6 and 10, groups 3 and 11, groups 4 and 12, groups 5 and 7, and groups 6 and 10.

HMNC containing dextrazides prepared from chemically oxidized dextrans. Lower values of phagocytosis by macrophages were observed for HMNC containing dextrazides derived from radiochemically oxidized dextrans, which can be explained by changes in lability of macrophage plasmalemma contacting with liposomal membranes of HMNC containing peroxide substances on their surface, which are formed during radiochemical exposure. They probably are responsible for lower stability of HMNC and more rapid dextrazide leakage from them [2,7,8]. The results of this study suggest that HMNC containing dextrazides synthesized from chemically oxidized dextran possess higher tropism to macrophages compared to HMNC containing dextrazides containing radiochemically oxidized dextrans and can be considered as a promising intracellular slow-release preparation for the therapy of tuberculosis.

The study was supported by Federal Target Program "Investigation and Development in Priority Areas of Scientific and Technological Complex Development in Russia in 2007-2012", Government contract No. 02.513.11.3183, April 23, 2007.

REFERENCES

1. E. D. Goldberg, A. M. Dygai, and V. P. Shakhov. *Methods of Cell Culture in Hematology* [in Russian], Tomsk (1992).
2. V. A. Sharpatiy, *Radiats. Biol. I Radioecol*., **39**, No. 1, 156-161 (1999).
3. V. A. Shkurupiy. *Tuberculous Granulomatosis. Cytophysiology and Targeted Drug Delivery* [in Russian], Moscow (2007).
4. V. A. Shkurupiy, S. A. Arkhipov, A. V. Troitsky, *et al*., *Bull. Exper. Biol. Med.*, **145**, No. 1, 123-126 (2008).
5. V. A. Shkurupiy, Yu. N. Kurunov, O. V. Grishin, *et al*., Method of Preparation of Slow-Release Isoniazide. Patent of Russian Federation No. 2143900, January 10, 2000, *Bull. Izobr. Polezn. Mod*., No.1 (2001).
6. V. A. Shkurupiy, Yu. N. Kurunov, and N. N. Yakovchenko, Lysosomotropism, Problems of Cell Biology and Medicine [in Russian]. Novosibirsk (1999).
7. V. A. Shkurupiy, S. A. Arkhipov, A. V. Troitsky, *et al*., *Bull. Exp. Biol. Med*., Suppl. 1, 120-122 (2008).
8. V. A. Shkurupiy, V. A. Krasnov, and E. P. Gulyaeva, *et al*., *Radiat. Phys. Chem*., **63,** 691-695 (2002).