Orthovanadate-based nanoparticles: differences in functional activity of cancer and hematopoietic stem cells

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Abstract—This research comparatively studies the influence of different concentrations of spherical gadolinium-yttrium orthovanadate nanoparticles ($GdYEuVO_4$) on a functional potential of Ehrlich carcinoma cancer and hematopoietic stem cells of healthy animals. Confocal microscopy data indicate the ability of nanoparticles at a concentration of 0.87 and 1.3 g / L to visualize the cells of tumor and those of healthy bone marrow; however, the intensity of their accumulation in myelokaryocytes was significantly lower than in Ehrlich carcinoma cells. It was shown that nanoparticles in both concentrations had the ability to inhibit the functional potential of both transformed and hematopoietic stem cells, but in the latter this was observed to a much lesser extent. Nanoparticles at a concentration of 1.3 g / L maximally reduced the metabolic activity of Ehrlich carcinoma cells and promoted an inhibition of tumor growth, while slightly altering the functional potential of hematopoietic stem cells.

Keywords—nanoparticles, orthovanadates, hematopoietic stem cells, cancer stem cells

I. Introduction

Advances in the development of nanotechnology indicate the possibility of using the nanoparticles (NPs) to develop the novel methods for diagnosis and treating various diseases. The fundamental possibility of visualization of bioobjects with the help of gadolinium-yttrium orthovanadate NPs ($GdYEuVO_4$), synthesized by the team of the Institute for Scintillation Materials of the National Academy of Sciences of Ukraine [1, 2], has been shown. It was previously established that only spherical NPs have the ability to a transmembrane penetration [1, 3]. In *vivo* research model of of Ehrlich carcinoma, the possibility of using NPs for the purpose of simultaneous targeting of cancer stem cells (CSCs) and inhibition of their function was substantiated [2, 4]. It has been proven that one of the possible ways of antitumor action of orthovanadate-based nanoparticles is generation of reactive oxygen species with subsequent damage to mitochondrial function or induction of cell death by apoptosis / necrosis [2, 4, 5]. This can nonspecifically affect function of different cell structures, that makes it relevant to study the biosafety of vanadium nano-sized compounds against hematopoietic cells of the recipient, especially stem cells, which have common features of their functioning with CSCs. The research was aimed to evaluate the influence of gadolinium-yttrium orthovanadate NPs $GdYEuVO_4$ displayed a spherical shape on functional potential of hematopoietic stem cells (HSCs) of healthy animals and Ehrlich carcinoma cells as well.

II. Materials and Methods

Ehrlich carcinoma (EC) cells, which were intraperitoneally injected into Balb / C mice, as well as one bone marrow (BM) cells of healthy CBA / H mice served as the research objects. The spherical orthovanadate nanoparticles (NPs) composed of $Gd_{0.6\text{-}0.8}Y_{0.1\text{-}0.3}VO_4:Eu^{3+}{}_{0.1}$ in various concentrations (0.87 g / L and 1.30 g / L) were obtained at the Institute for Scintillation Materials of the National Academy of Sciences of Ukraine (Kharkiv) by means of colloidal synthesis [6]. Aqueous colloidal solutions of orthovanadate NPs were purified from impurities by dialysis using Cellu Sep H1 3.5 KDa membranes. The sizes and morphology of NPs were assessed using transmission electron microscopy (TEM) with a TEM 125K electron microscope (Selmi, Ukraine) at an accelerating voltage of 100 kV (magnification up to × 100000). For studies in the transmission mode, the samples were prepared according to the standard technique by applying a colloidal solution to copper grids covered with a thin layer of carbon, followed by drying them at room temperature.

The studied cells were incubated with NPs for 3 hours, after that their metabolic activity was assessed by the MTT test using the "Cell growth determination kit, MTT based" (Stock No. CGD-1, Sigma, USA) according to the manufacturer's instructions. The results were detected by measuring the optical density of the samples using a spectrophotometer Lambda 465 (PerkinElmer, USA) at a wavelength of 540 nm.

NPs were visualized after incubation with BM and EC cells by confocal laser scanning microscopy LSM 510 META

(Carl Zeiss, Germany). The functional activity of EC cells after NP pretreatment was assessed on day 7 after their intraperitoneal injection at a concentration of $3x10^6$ cells / mouse according to the following parameters:

- absolute number of cells (ANC) according to the formula: ANC = Vol x Conc, where Vol is the volume of ascitic fluid in the peritoneal cavity (PC) of animals (ml), Conc is the concentration of tumor cells, which were counted in the Goryaev's chamber ($x10^7$ cells/ml).
- tumor growth intensity (TGI) according to the formula: TGI = (ANC (experiment) / ANC (control)) x 100%,

where ANC (experiment) - the absolute number of tumor cells in the PC of animals with EC, initiated by cells pretreated with NPs; ANC (control) - the absolute number of cells in the PC of animals with EC, initiated by cells untreated with NPs.

Functional activity of bone marrow HSCs after NP pre-treatment was determined by their ability to form colonies in spleen on day 8 after injection to lethally irradiated recipients as reported by J.E. Till and E.A. McCulloch [7]. Mice were irradiated by means of "RUM-17" device at a dose of 8 Gy. One hour after irradiation, the mice were injected into the tail vein with BM cells at a dose of $1x10^5$ cells / mouse in 0.2 ml of saline. The control group animals were injected with BM cells without NP treatment at the same dose. The number of BM cells in observed mice was examined on day 8 after irradiation. In mice, the thigh was removed, BM was washed out from them in 1 ml of handling medium and the number of cells in Goryaev's chamber was counted and their content in a thigh was counted.

To assess the effect of NPs on functional activity of CSCs the rate of tumor growth inhibition (RTGI) was calculated by the formula:

RTGI = TGI (control) - TGI (exp),

where TGI (control) - tumor growth intensity of the control group, TGI (exp) - tumor growth intensity of the experimental group. The tumor growth intensity of EC cells without pre-treatment of NPs served as a control and was assumed as 100%.

Rate of colony formation inhibition was calculated by the formula:

(nCFUs(control)-CFUs(exp)): nCFUs(control) x 100%,

where CFUs (control) is the number of colonies in spleen of irradiated recipients of the control group, CFUs (exp) is the number of colonies in spleen of irradiated recipients of experimental group.

Rate of metabolic activity inhibition of EC and BM cells (MTT- test) was calculated by the formula:

((Metabolic activity (control) - Metabolic activity (exp)): Metabolic activity (control) x 100%.

The data obtained were statistically processed using the STATISTIKA 8.0 software. The obtained values are expressed as the mean ± SD. In the case of independent samples, the Mann-Whitney U-test was used, and for dependent ones we applied the Wilcoxon W-test. Differences were considered significant at $p < 0.05$.

III. Results and Discussion

The investigated aqueous solutions of NPs, as typical colloidal systems, were transparent in transmitted light and opalescent under lateral illumination (Tyndall cone was observed).

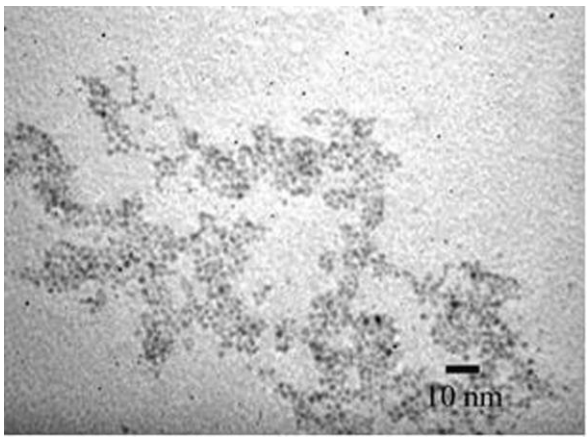

Fig. 1. TEM image of solid phase of spherical nanoparticles ($GdYEuVO_4$) aqueous solution.

Under UV irradiation, aqueous solutions of NPs exhibited a bright red luminescence. Their pH value was within the range of 7.4-7.8. Colloidal solutions of NPs easily passed through nitrocellulose ultrafilters with a pore diameter of 100 nm. The concentration of the solid phase of aqueous solutions of NPs was 0.87 and 1.3 g / L. Sterilization of NP solutions at 120° C for 1-2 hours in sealed ampoules did not cause sedimentation and coagulation of colloids.

The TEM method showed that the NPs were of a spherical shape with an average diameter of 2–3 nm (Fig. 1). The size dispersion did not exceed 15%.

By means of confocal microscopy, it was found that after 3 hours of incubation, NPs penetrated into all tumor cells. Regardless of the concentration of the NPs used, the mean fluorescence intensity of EC cells was 177.3 ± 24.5 arb. units (Fig. 2).

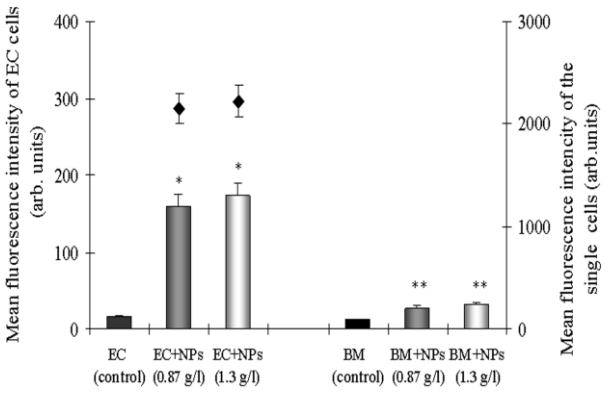

Fig. 2. Mean fluorescence intensity of total population of EC and BM cells (rectangular columns) and fluorescence of single cells (marker) after incubation with NPs of various concentrations. * - difference is significant in comparison with the similar indices of EC and BM cells without incubation with NPs (control) ($p < 0.05$).

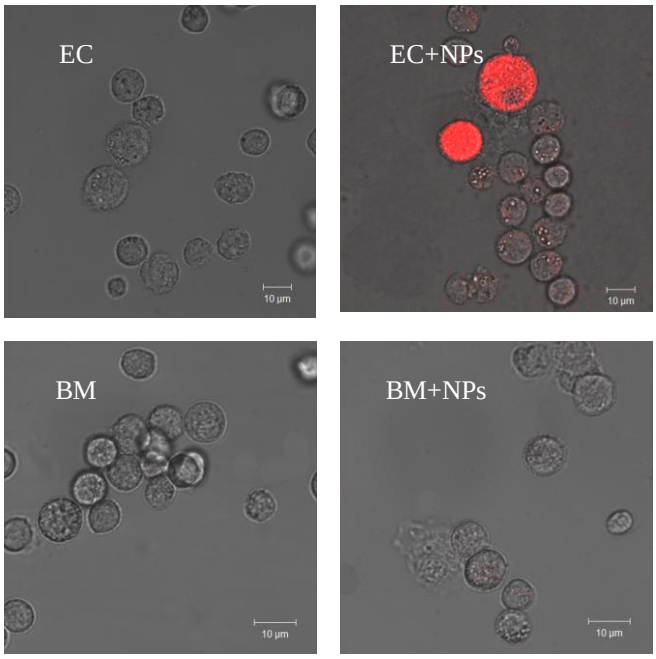

Fig. 3. Confocal microscopy of EC and BM cells before and after incubation with NPs.

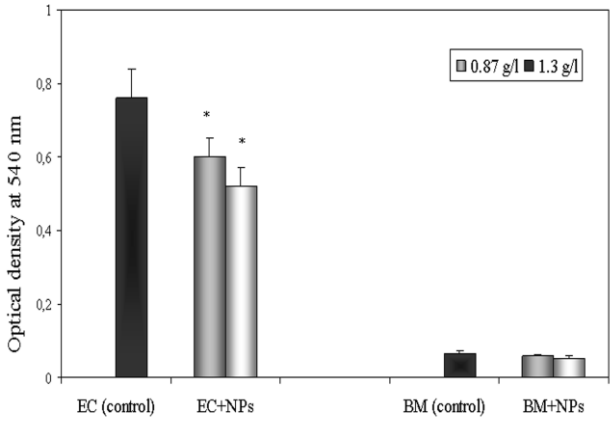

Fig. 4. Metabolic activity of EC and BM cells before and after incubation with NPs. * - difference is significant compared with similar indices of EC and BM cells without incubation with NPs (control) ($p < 0.05$).

In control samples without NP treatment, this value did not exceed 15.3 ± 3.3 arb. units, which corresponds to the level of the detector electronic noise. At the same time, single tumor cells had an ultra-high level of luminescence intensity (2,220.5 ± 119.5 arb. units), the number of such cells did not exceed 3% (4 per 150 cells) (Figs. 2 and 3). Previously, it was demonstrated that this subpopulation of EC cells was simultaneously capable of intense staining with monoclonal antibodies to the CD44 antigen [4], which indicates their belonging to CSCs.

Unlike tumor cells, the mean fluorescence intensity of which was 177.3 ± 24.5 arb. units, myelokaryocytes were able to accumulate NPs to a lesser extent, and after 3 hours of incubation their fluorescence intensity made 31.0 ± 12.8 arb. units (Fig. 2, 3) (control demonstrated 11.6 ± 3.5 arb. units).

The results obtained indicate that NPs have a selective ability to increase the accumulation in tumor cells, in contrast to BM ones, which accumulate them to a small extent.

According to the MTT test results, it was found that NPs at the concentrations used in a dose-dependent manner reduced the metabolic activity of EC cells: pretreatment with NPs at a concentration of 0.87 g / L - by 21.3 ± 0.7%, and 1.3 g / L - by 31.8 ± 1.4%, respectively (Fig. 4). The inhibitory effect of NPs on the metabolism of BM cells was less significant, i.e. the NPs at a concentration of 0.87 g / L and 1.3 g / L reduced the activity of mitochondrial respiration by 18.7 ± 1.5 and 23.1 ± 2.1%, respectively. The findings show that the inhibition of mitochondrial respiration, which reflects the state of mitochondrial enzymatic system, occurs more intensely in EC cells than in BM ones. This is an early sign of toxic effects of NPs, which can subsequently lead to the death of tumor cells.

This assumption was experimentally confirmed *in vivo* by estimating the intensity growth of EC cells that had been previously incubated with NPs (Table 1). Under the influence of NPs of both concentrations there was a decrease in the intensity of tumor development, judging by the absolute number of EC cells in PC (Table 1). However, only the use of NPs at a concentration of 1.3 g / L led to a significant decrease in the absolute number of EC cells in PC (23.03 ± 4.87%) compared with the control (55.09 ± 7.56%).

In another experimental model, namely in determining the content of colony-forming units of the spleen (CFUs) in lethally irradiated animals after administration of BM cells, it was found that different concentrations of NPs almost equally inhibited the colony-forming activity of BM cells, as evidenced by a decrease in CFUs in spleen of lethally irradiated recipients (Table 2). The dependence of bone marrow hematopoiesis on the concentration of NPs, judging by the number of BM per a thigh (Table 2) has been revealed.

TABLE I. INTENSITY GROWTH OF EC CELLS, PRETREATED WITH NPS OF DIFFERENT CONCENTRATIONS

Group	Index			
	Volume of ascitic fluid, ml	***Number of EC cells in PC, x10^7***	***Absolute number of EC cells in PC, x10^7***	***Tumor growth intensity, %***
Control (EC cells)	5.8±0.8	9.5±1.6	55.1±7.6	100
EC+NPs (0.87 g/L)	4.4±0.9	10.2±1.4	44.9±6.7	81.50±4.4
EC+NPs (1.3 g/L)	3.1±1.0	7.5±2.1	23.0±4.9[a]	41.80±3.5

[a.] difference is statistically significant compared with similar indices of EC cells without incubation with NPs (control) ($p < 0.05$).

TABLE II. NUMBER OF BM CELLS PER THIGH AND FORMED COLONIES (CFUS) IN IRRADIATED MICE ON DAY 8 AFTER INJECTION OF BM CELLS, PRETREATED WITH NPS OF DIFFERENT CONCENTRATIONS

Group	Index	
	The number of BM cells in a thigh, x10^5	***Number of colonies in spleen (CFUs), abs.units***
Control (BM cells)	13.00±0.8	15.0±1.5
BM+NPs (0.87 g/L)	11.5±0.6	14.0±1.3
BM+NPs (1.3 g/L)	9.8±0.7	13.5±2.0

To assess the differences in the effects of NPs of different concentrations relative to transformed and normal stem cells, the rate of inhibition of functional activity of EC and BM cells was calculated (Table 3).

TABLE III. RATE OF INHIBITION OF FUNCTIONAL INDICES OF EC AND BM CELLS PRETREATED WITH NPS OF DEFFERENT CONCENTRATIONS

Concentrations of NPs, g/L	Index			
	Rate of tumor growth inhibition, %	***Rate of metabolic activity inhibition of EC cells, %***	***Rate of colony formation inhibition (CFUs), %***	***Rate of metabolic activity inhibition of BM cells, %***
0.87	18.0±1.3	21.3 ±0.7	18.7±1.5	10.7 ±0.3
1.3	58.24±3.5	31.8 ±1.4	23.1±2.1	18.5 ±0.6

[a.] difference is statistically significant compared with similar indices of EC cells without incubation with NPs (control) ($p < 0.05$).

The NPs of both concentrations were shown to reduce the functional potential of both transformed tumor cells and hematopoietic stem cells, but the latter to a much lesser extent. Nanoparticles at a concentration of 0.87 g / L to the same extent inhibited both tumor growth and colony-forming activity of HSCs (18%). The rate of tumor growth inhibition in the pretreatment EC cells with a higher concentration of NPs (1.3 g / L) was a maximum (58.24 ± 3.5%), which is three times higher than that when using 0.87 g / L NPs. At the same time, the increase in the NPs concentration did not strongly affect the functional activity of HSCs, judging by the number of CFUs in this group.

IV. CONCLUSIONS

The data obtained indicate that the gadolinium-yttrium orthovanadate NPs ($GdYEuVO_4$) due to their versatility are the new theranostic agents to be used in cancer therapy, while having the ability to visualize the cancer cells and inhibit their function. NPs at a concentration of 1.3 g / L are able to extremely alter the functional activity of CSCs, but they are not indifferent structures to normal stem cells, in particular HSCs.

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