Experimental Study of the Effects of Metal Nanoparticles on Tumor Growth and Bone Marrow Hemopoiesis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 153, No. 1, pp. 113-117, January, 2012
Original article submitted August 17, 2010

Metal (Zn, Cu, Fe) nanoparticles, particularly Zn, induce regression of transplanted sarcoma 37 and increase the percentage of dead and degenerated cells. Antitumor effect of metal nanoparticles was not associated with leukopenia or coarse bone marrow abnormalities, but differential leukocyte and bone marrow counts remained changed.

Key Words: *metal nanoparticles; sarcoma 37; tumor cells; blood; bone marrow*

Nanotechnologies is an innovation research trend; some of its aspects can be used in biology and medicine [4,6,10], for example, in oncology [7-9,11]. Studies of biotropic characteristics of nanoparticles (NP) are now in progress [1]. Macro- and trace elements are essential for vital function support; they are involved in metabolic processes and homeostasis regulation [5]. Iron, copper, and zinc are elements essential for human organism. On the other hand, metals and their compounds inhibit cell growth. Some of them (for example, platinum) are components of cytostatics used for the treatment of malignant tumors. Metals in the form of NP exhibit high biological activity and are long acting and less toxic than their salts. Reduction of particle size to the nanometer range improves their penetration and reaction capacity and leads to emergence of the quantum size effects [10]. Cytotoxic and antiproliferative effects described for some metal NP [1-3,7,8] have been described, as well as the possibility of their side effects.

We carried out an experimental study of metal NP effects on tumor growth and hemostasis.

MATERIALS AND METHODS

Nanoparticles (300-1000 Å) used in the study were ultradispersed metal powders (Cu, Zn, Fe) synthesized at Saratov Plasmochemical Complex. Experimental studies were carried out on mouse continuous ascitic sarcoma 37 cells (passages 1-2) from the collection of N. N. Blokhin Cancer Research Center. Experiments were carried out on outbred male mice (18-20 g; n=40) kept under standard vivarium conditions. Sarcoma 37 was allowed to grow for 4 days after intraperitoneal transplantation, after which experimental animals were daily injected intraperitoneally with 0.5 ml of the metal (Zn, Cu, or Fe) NP suspension in a concentration of 10 μg/ml. Controls were similarly injected with 0.5 ml isotonic NaCl. NP were injected for 4 days, the total dose was 20 μg/mouse (1 mg/kg), after which the animals were observed for 4 days. On day 13 after tumor transplantation the ascitic fluid (AF) in each mouse was removed with a syringe. AF volume was measured, tumor cells were counted in a Goryaev chamber, the percentage of dead cells was evaluated by trypan blue staining. The number of viable tumor cells per ml AF was evaluated. The solid component of the tumor developing in the abdominal cavity was measured, fixed in formalin, stained with hematoxylin and eosin, and examined under a light microscope. The blood was collected for evaluation of differential leukocyte count. Bone marrow was collected from the femoral bones for evaluation of differential cell count.

The data were statistically processed using parametric Student's test and nonparametric Wilcoxon's and z sign tests.

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0007-4888/12/1531-0101

RESULTS

The volume of AF in the abdominal cavity was minimum in animals injected with Zn NP and maximum in those injected with Fe NP (Table 1). Only in mice injected with Zn NP, the volume of AF was statistically lower than in the control. AF volume in this experimental group was lower than in mice injected with Cu and Fe NP. Similar differences were found for the counts of tumor cells in AF. The count of viable tumor cells (per ml) AF was minimum in animals injected with Zn NP and maximum in controls. Cell counts were similar in AF from mice treated with Cu and Fe NP and significantly lower than in controls but higher than in mice treated with Zn NP.

Solid component of sarcoma 37 was also minimum in mice injected with Zn NP (Tables 1, 2). The tumor capsule in mice treated with Zn NP was significantly thicker than in controls. Vast necrotic fields were found between the foci of tumor cells (Fig. 1, *b*) and fatty degeneration (Fig. 1, *c*), while large foci with dense or loosely packed tumor cells were seen in controls (Fig. 1, *a*). Injection of Cu NP led to similar changes (Fig. 1, *d*, *e*). Necrotic foci were negligible in animals injected with Fe NP; the size and type of tumor foci were close to those in controls (Fig. 1, *f*).

TABLE 1. Effects of Metal NP on Sarcoma 37 Growth in Mice

Group	Volume		Count of tumor cells/ml AF
	AF, ml	solid tumor, mm^3	
Control	1.79±0.36	574±95	27.70±2.86
Zn NP	0.78±0.31*	154±88*	1.67±0.50*
Cu NP	2.16±0.68^{+}	374±90	8.13±1.54^{*+}
Fe NP	3.3±1.0^{+}	322±112	8.26±2.37^{*+}

Note. $p<0.05$ in comparison with: *control, $^{+}$Zn NP.

Sarcoma 37 cells were presented by two generations: large polygonal cells with oxyphilic cytoplasm and small oval cells with basophilic cytoplasm, each type forming different foci. Cells of the former generation predominated in animals injected with Fe NP, while the latter generation predominated in mice injected with Cu NP. There were also giant cells: up

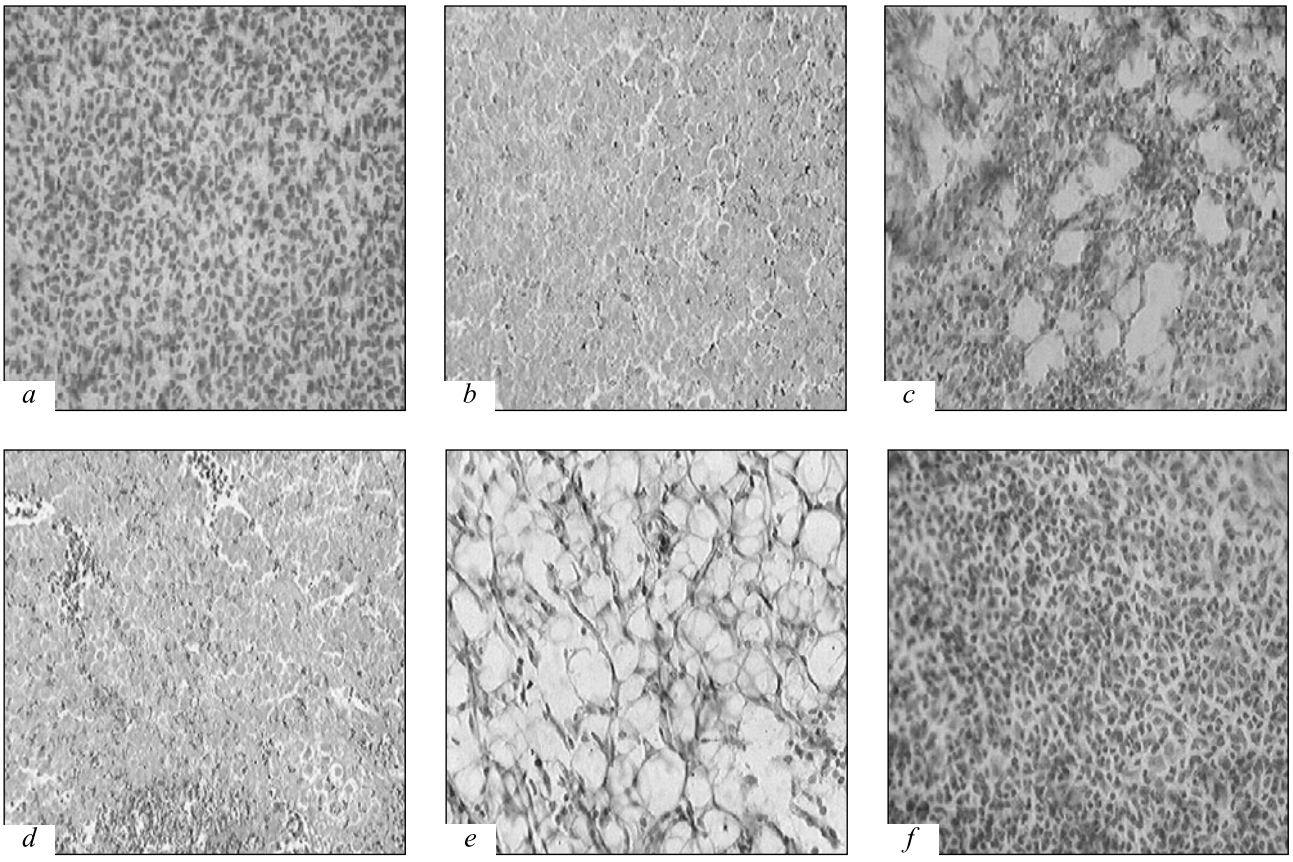

Fig. 1. Microscopic characteristics of sarcoma 37 in experimental and control mice. Hematoxylin and eosin staining, ×400. *a*) control; *b*) Zn NP, necrosis field; *c*) Zn NP, fatty degeneration; *d*) Cu NP, necrosis field; *e*) Cu NP, fatty degeneration; *f*) Fe NP.

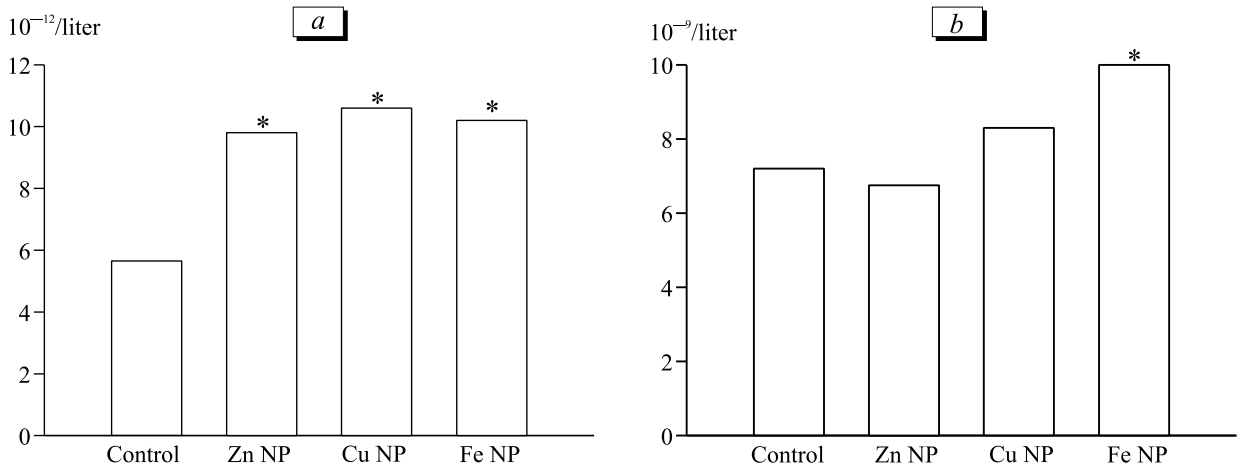

Fig. 2. Effects of metal NP on the levels of erythrocytes (*a*) and leukocytes (*b*) in mice with sarcoma 37. $p<0.05$ in comparison with the control.

to 20 per visual field in the control, 17 after Fe NP, 10 after Cu NP, and up to 8 per visual field in mice injected with Zn NP. The number of tumor cells in mitosis was also the minimum in animals treated with Zn NP - 1-2 per visual field, *vs.* 3-4 in animals injected with Cu NP and 6-7 in those injected with Fe NP (this value was in fact similar to the control).

Injections of Zn and Cu NP caused a drastic increase in the percentage of degenerative tumor cells, the effect of Zn NP being more pronounced (Table 2). The predominant type of degeneration was karyolysis (the most coarse injuries), while karyopyknosis and vacuolation were less included than in other groups. Hence, injections of Zn NP promoted greater injuries to sarcoma 37 cells, Cu NP ranked second, while the effect of Fe NP was the minimum.

Analysis of metal NP effects on the blood cell composition in animals with tumors showed a significant (1.5-2-fold) increase of erythrocyte levels in mice injected with all the studied metal NP (Fig. 2, *a*). Injections of metal NP did not cause leukopenia: leukocyte level in the peripheral blood of mice treated with Zn and Cu NP did not differ from the control, while injections of Fe NP promoted an increase of their level from 7.17±0.84 to (10.0±1.89)×10^{-9}/liter ($p<0.05$; Fig. 2, *b*). The differential leukocyte count in animals of experimental groups were different (Fig. 3). Tumor development was paralleled by an increase in the count of segmented neutrophils from 20.4±1.95 to 39.2±3.3 ($p<0.05$) in parallel with reduction of lymphocyte level from 77.8±1.84 to 58.8±3.4% in comparison with that in intact animals ($p<0.05$). Injection of Cu NP to animals with tumors led to a slight elevation of segmented neutrophils and reduction of lymphocyte level; injections of Zn and Fe NP induced opposite changes. Though these differences were negligible in comparison with the values in control animals with tumors, the changes between the groups of mice injected with different NP were statistically significant. Lymphocyte levels were higher in mice injected with Zn and Fe NP (64.4±2.2 and 64.5±2.9%; $p<0.05$), while the levels of segmented neutrophils were lower (35.2±2.0 and 34.3±2.8%; $p<0.05$) than after injections of Cu NP (52.5±3.8 and 46.3±3.6%, respectively). Injections of Fe and Zn NP caused a reduction of blood levels of stab neutrophils in comparison with the control and with animals injected with Cu NP. These cells were not found in 5 of 10 mice injected with Cu NP, in 9 of 10 injected with Zn NP, and in 7 of 10 injected with Fe NP, while in the control group they were found in 7 animals. No eosinophils were found in the peripheral blood of mice injected with metal NP, in contrast to controls.

Studies of the bone marrow from experimental and control mice showed no coarse injuries characteristic of the cytostatic effect. Injections of Cu NP caused no statistically significant differences in differential bone marrow cell count in comparison with the control.

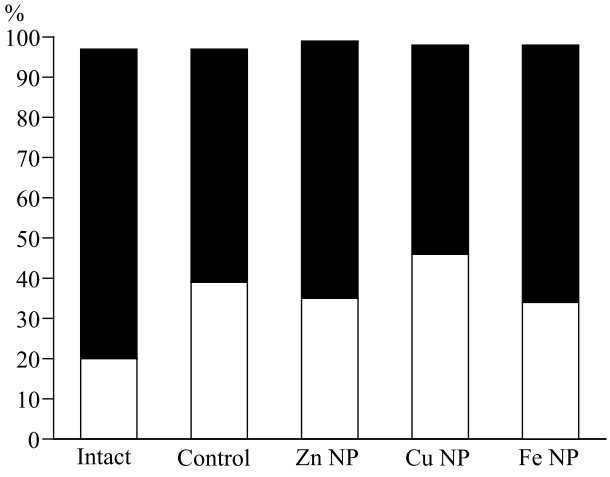

Fig. 3. Effects of metal NP on the levels of segmented neutrophils (light bars) and lymphocytes (dark bars) in mice with sarcoma 37.

TABLE 2. Comparative Characteristics of Degenerative Changes in Sarcoma 37 Cells after Injections of Different Metal NP

Group	Degenerative cells, %	Types of degenerative changes, %			
		karyopyknosis	karyorhexis	karyolysis	vacuolation of nucleus and cytoplasm
Control	13.74±2.30	22.53±1.18	29.41±1.79	29.41±1.79	17.65±1.52
Zn NP	71.69±8.74*	16.67±1.50*	29.17±2.02	50.00±6.11*	4.16±0.73*
Cu NP	48.20±5.58*	21.43±1.88	28.57±3.37	42.86±3.20*	7.14±1.05*
Fe NP	21.66±4.31	17.65±2.22	35.29±4.35	29.41±2.78	17.65±1.32

Note. *$p<0.05$ in comparison with the control.

Injections of Zn NP caused a significant reduction of myelocyte level (4.23±0.21% *vs.* 5.22±0.38% in the control) and suppression of the lymphoid stem (lymphocyte and lymphoblast level was 7.74±0.81% *vs.* 10.06±0.71% in the control), including plasma cells not found in mice of this group. Reduction of lymphoid cell level in the bone marrow could result from their release into the blood and/or reaction to pronounced regression of the tumor. Injection of Fe NP led to suppression of the myeloid and eosinophilic lineages and of mast cells; the level of erythroid precursors increased by 40% in comparison with the control. On the whole, we can hypothesize suppression of the cells mediating the immune reactions, mainly humoral, under the effect of Zn NP and of those mediating allergic reactions under the effect of Fe NP.

Hence, experiments on mice with transplanted sarcoma 37 showed the maximum destructive effect on the tumor of Zn NP, a less pronounced effect of Cu NP, and minor effect of Fe NP. This antitumor effect was associated with changes in the white blood picture (most pronounced under the effect of Cu NP) and the bone marrow (most pronounced under the effect of Zn NP). Together with our previous experimental data on the absence of thymus injuries and even its stimulation under the effect of Zn NP [2], these facts suggest that the T-cell immunity (playing the key role in antitumor immunity) is retained, while suppression of the lymphoid cells observed in our experiments should be referred mainly to B-cells. In contrast to the cytostatics, the antitumor effect of metal NP in the studied doses was not associated with myelosuppression and hematological toxicity.

The authors are grateful to Prof. V. V. Borodulin, Saratov Medical University, for his kind gift – metal nanoparticles.

REFERENCES

1. I. P. Arsentyeva, E. S. Zotova, G. E. Folmanis, *et al.*, *Nanotekhn.*, No. 2, 72-77 (2007).
2. E. Yu. Zlatnik, L. V. Peredreyeva, and I. A. Goroshinskaya, *Sib. Onkol. Zh.*, Suppl. No. 2, 81 (2009).
3. E. Yu. Zlatnik and G. I. Zakora, *Ros. Allergol. Zh.*, No. 3, Issue No. 1, 201-202 (2009).
4. A. V. Ivanov V. M. Mushta, V. G. Pevgov, *et al.*, *Ros. Bioterapevt. Zh.*, **6**, No. 1, 74 (2007).
5. A. V. Kudrin and O. M. Gromova, *Trace Elements in Immunology and Oncology* [in Russian], Moscow (2007).
6. V. M. Lakhtin, S. S. Afanasyev, M. V. Lakhtin, *et al.*, *Vestn. Rossiisk. Akad. Med. Nauk*, No. 4, 50-55 (2008).
7. Yu. P. Meshalkin and N. P. Bgatova, *J. Siberian Federal University*, **1**, No. 3, 204-208 (2008).
8. N. Ya. Rapoport, *Vestn. Rossiisk. Akad. Med. Nauk*, No. 4, 7-8 (2008).
9. V. F. Chekhun, *Onkologiya*, **10**, No. 1, 7-8 (2008).
10. M. I. Baraton, *Synthesis, Functionalization, and Surface Treatment of Nanoparticles*, Los Angeles (2002), pp. 234-236.
11. S. Nie, Y. Xing, G. J. Kim, and J. W. Simons, *Ann. Rev. Biomed. Eng.*, **9**, 257-288 (2007).