NANOTECHNOLOGY

Modulation of Oxygen-Dependent and Oxygen-Independent Metabolism of Neutrophilic Granulocytes by Quantum Points

S. N. Pleskova*, and E. R. Mikheeva*,****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 4, pp. 452-454, April, 2011
Original article submitted March 21, 2010

Inhibition of neutrophilic granulocyte metabolism under the effect of semiconductor quantum points was demonstrated. The status of the oxidative system was evaluated by the NBT test, nonoxidative status by the lysosomal cationic test. It was found that quantum points in a dose of 0.1 mg/ml irrespective of their core and composition of coating significantly inhibited oxygen-dependent and oxygen-independent metabolism of neutrophilic granulocytes.

Key Words: *quantum points; neutrophilic granulocytes*

The mechanisms of nonspecific organism's resistance in humans are inevitably activated in response to foreign substances entering the inner media [5]. However, absorption of nanomaterials, including quantum points, and stimulation of nonspecific defense caused by them have some specific features. For example, nanomaterials are positively toxic towards various kinds of tissue cultures, including cellular component of nonspecific resistance [4,7]. Induction of oxidative stress by quantum points can be one of the main causes of their toxicity [6]. It is of crucial importance for neutrophilic granulocytes, because production of oxygen radicals by these cells is the molecular basis of realization of their effector function [2]. Hence, stimulation of the respiratory burst together with the exogenous source of active oxygen forms will inevitably cause failure of oxygen-dependent cell metabolism and modify nonoxidative killing.

We studied changes in oxygen-dependent and oxygen-independent metabolism of neutrophilic granulocytes under the effects of quantum points.

*Physics of Solid-State Nanostructures Research and Education Center, N. I. Lobachevskii State University, Nizhnii Novgorod; **R. E. Alekseev State Technological University, Nizhnii Novgorod, Russia. ***Address for correspondence:*** pleskova@mail.ru. S. N. Pleskova

MATERIALS AND METHODS

Neutrophilic granulocytes were isolated from the venous blood of healthy donors by fractionation of heparin-treated blood in double Ficoll-Verograffin gradient, after which the cells were collected and washed in buffered saline. Neutrophilic granulocytes were suspended in Hanks saline (M. P. Chumakov Institute of Poliomyelitis and Viral Encephalitides). Cells in a final concentration of 2×10^6/ml were used in experiments.

Neutrophil metabolism was modulated by quantum points of several types: CdSe/ZnS-mercaptopropionic acid (maximum emission at 604 nm), (CdSe/CdZnS)ZnS-polyT (maximum emission at 626 nm), CdSeCdSZnS/polyT/SiO_2-NH_2 (maximum emission at 590 nm), synthesized at Nanotech-Dubna Center, and CdSe/CdS-mercaptoacetic acid (maximum emission at 580 nm), synthesized at laboratory headed by V. R. Zlomanov (M. V. Lomonosov Moscow University). According to specification, the size of quantum points varied from 9 nm (minimum size) to 30 nm (maximum size), 90% quantum points being of 11-20 nm in size. In order to evaluate the effects of quantum points on oxygen-dependent and oxygen-independent metabolism of neutrophilic granulocytes, they were added to

0007-4888/11/1514-0470

TABLE 1. Inhibition of Respiratory Activity of Neutrophilic Granulocytes under the Effect of Quantum Points with Different Core and Coating Composition (Final Concentration of Quantum Points 0.1 mg/ml)

Neutrophils	Control	(CdSe/CdZnS)ZnS-polyT	CdSe/ZnS-mercaptopropionic acid	CdSeCdSZnS/polyT/SiO_2-NH_2
NBT-positive in induced test	40.5±12.4	20.3±4.3* (t=3.75; *n*=12)	24.5±3.9* (t=3.0; *n*=12)	23.2±3.7* (t=3.27; *n*=12)
NBT-positive in spontaneous test	12.8±0.9	8.8±0.8* (t=7.91; *n*=12)	6.8±2.6* (t=5.35; *n*=12)	7.7±1.8* (t=6.3; *n*=12)

Note. Here and in Table 2: *p<0.05 compared to the control.

the system in a final concentration of 0.1 mg/ml, for studies of the dose-dependent effect in different final concentrations.

NBT test was carried out as described previously [1] and the lysosomal cationic test (LCT) by Pigarevsky's method [3].

The results were analyzed using Origin 5.0 Server software.

RESULTS

The results of inhibition of oxygen-dependent metabolism of neutrophilic granulocytes under the effect of quantum points (final concentration 0.1 mg/ml) according to induced (opsonized zymosan) and spontaneous NBT tests are summed up in Table 1.

Incubation with semiconductor nanoparticles for 60 min significantly suppressed both the basal level of cellular respiratory activity and respiratory activity of neutrophilic granulocytes during the respiratory burst. Stimulated cells are more sensitive to quantum points. It seems most likely that oxidative activity of neutrophils in that case is potentiated by the effects of active oxygen forms produced with active participation of the nanoparticles. The data (Table 1) demonstrate that the oxygen-dependent component of neutrophilic granulocyte metabolism is disordered in any case, irrespective of chemical composition of the quantum points. However, the CdSe/CdS nanocrystals coated with mercaptoacetic acid exhibited the highest activity. These quantum points (in a final concentration of 0.1 mg/ml) caused 100% cell death in spontaneous and induced NBT test during 60-min incubation. Hence, in order to evaluate the dose-dependent inhibition of the neutrophil oxidative activity, experiments with different concentrations of CdSe/CdS-mercaptoacetic acid were carried out (Table 2).

The data (Table 2) reflect dose-dependent inhibition of spontaneous and induced respiratory activity of neutrophilic granulocytes. Further increase in the dose of nanoparticles led to cell destruction, and evaluation of the NBT test results at quantum points (CdSe/CdS-mercaptoacetic acid) concentration of 0.07 mg/ml and higher was impossible.

Since the neutrophil killing function under conditions of their disordered oxygen-dependent metabolism can be partially compensated for by activity of the bactericidal system, we studied the effects of nanoparticles on the granular system of neutrophilic granulocytes. The results of LCT showed that the mean cytochemical coefficient (MCC) in the control was 1.37±0.4, while after incubation with nanoparticles it decreased significantly. MCC was 0.88±0.62 (t=12.52; *n*=1200; p<0.05) for (CdSe/CdZnS)ZnS-polyT, 0.98±0.60 (t=11.94; *n*=1200; p<0.05) for CdSeCdSZnS/polyT/SiO_2-NH_2, and 0.89±0.68 (t=11.94;

TABLE 2. Dose-Dependent Inhibition of Respiratory Activity of Neutrophilic Granulocytes under the Effect of CdSe/CdS-Mercaptoacetic Acid (Exposure 60 min)

Neutrophils	Control	Dose, mg/ml				
		0.02	0.03	0.04	0.05	0.06
NBT-positive in induced test	37.9±6.6	31.5±0.7 (t=1.34; *n*=22)	25.8±3.9* (t=3.5; *n*=24)	24.8±2.1* (t=3.9; *n*=24)	19.3±2.2* (t=5.5; *n*=24)	17.3±2.9* (t=6.0; *n*=24)
NBT-positive in spontaneous test	13.1±2.8	8.7±2.1* (t=3.5; *n*=28)	8.3±1.7* (t=3.3; *n*=26)	7.3±1.7* (t=3.9; *n*=26)	6.5±1.3* (t=4.6; *n*=26)	5.8±1.7* (t=5.0; *n*=26)

n=1200; p<0.05) for CdSe/ZnS-mercaptopropionic acid.

Hence, inhibition of cell metabolism was an obvious result of the effect of quantum points on human neutrophilic granulocytes. Quantum points with different chemical structure of the core and envelope caused failure of the neutrophil oxidative system and a significant reduction of the cellular secretory potential. Starting from the concentration of 0.03 mg/ml, the CdSe/CdS-mercaptoacetic acid quantum points caused a statistically significant reduction of the parameters of spontaneous and induced NBT-test.

The authors are grateful to I. V. Balalaeva for CdSe/CdS-mercaptoacetic acid quantum points and to A. A. Brilkin for NBT reagent.

The study was supported by the Russian Foundation for Basic Research (grant No. 09-04-97068-r_povolzhye_a) and FTP "Scientific and Research and Pedagogical Workers of Innovation Russia" (SC No. 14.740.11.0456).

REFERENCES

1. I. G. Gerasimov and D. Yu. Ignatov, *Tsitologiya*, **46**, 155-158 (2004).
2. V. M. Mushtakova, V. A. Fomina, and V. V. Rogovin, *Byull. Eksp. Biol. Med.*, **147**, No. 6, 647-649 (2009).
3. V. E. Pigarevskii, *Clinical Morphology of Neutrophilic Granulocytes* [in Russian], Leningrad (1988), pp. 87-101.
4. G. Guo, W. Liu, J. Liang, *et al.*, *Materials Lett.*, **61**, Nos. 8-9, 1641-1644 (2007).
5. R. Medzhitov and Ch. Janeway, *N. Engl. J. Med.*, **343**, No. 3, 338-344 (2000).
6. A. Nel, T. Xia, L. Mädler, and N. Li, *Science*, **311**, 622-627 (2006).
7. A. Shiohara, A. Hoshino, K. Hanaki, *et al.*, *Microbiol. Immunol.*, **48**, No. 9, 669-675 (2004).