The study of indicators of bone marrow and peripheral blood of rats with diabetes and transplanted liver tumor after intravenous injection of gold nanorods

Natalya I. Dikht[a], Alla B. Bucharskaya*[a], Galina N. Maslyakova[a], Georgy S. Terentyuk[ab] , Olga V. Matveeva[a], Nikita A. Navolokin[a], Boris N. Khlebtsov[c], Nikolai G. Khlebtsov[c]
[a]Saratov State Medical University, 112 B.Kazachya str., Saratov, Russia; [b] Saratov State University, 83 Astrakhanskaya str., Saratov, Russia; [c]Institute of Biochemistry and Physiology of Plants and Microorganisms RAS, 13 Entuziastov Ave., Saratov, Russia

ABSTRACT

In study the evaluation of the influence of gold nanorods on morphological indicators of red bone marrow and peripheral blood of rats with diabetes and transplanted liver tumor after intravenous administration of gold nanorods was conducted. We used gold nanorods with length 41 ± 8 nm and diameter of 10.2±2 nm, synthesized in the laboratory of nanobiotechnology IBPPM RAS (Saratov). After intravenous administration of gold nanorods the decrease of leukocytes, platelets and lymphocytes was observed in animals of control group in blood. It was marked the decrease of the number of mature cellular elements of the leukocyte germ in bone marrow - stab neutrophils and segmented leukocytes, and the increase of immature elements- metamyelocytes, indicating the activation of leukocyte germ after nanoparticle administration. The decrease of leukocyte amount was noted in blood and the increase of cellular elements of the leukocyte germ was revealed in bone marrow, indicating the activation of leukocyte germ in rats with alloxan diabetes and transplanted tumors. The changes of morphological indicators of blood and bone marrow testify about stimulation of myelocytic sprouts of hemopoiesis in bone marrow as a result of reduction of mature cells in peripheral blood after gold nanoparticle administration.

Keywords: gold nanorods, diabetes, transplanted liver tumor, blood indicators, bone marrow

1. INTRODUCTION

The diabetes mellitus takes the fourth place among the causes of premature mortality. Recent estimates of World Health Organization indicate that there were 171 million people in the world with diabetes in 2000 and it is projected to increase to 366 million in 2030[1]. Large number of epidemiological studies have demonstrated an association between increased risk of cancer development and progression and type 2 of diabetes mellitus as well as obesity[2, 3].

The lack of effectiveness of therapy methods of diabetes and malignant tumors causes the search of new treatment technologies. The active use of nanotechnologies in diagnostics and treatment of different diseases and pathological processes is currently one of the promising areas of its biomedical applications[4]. The considerable amount of scientific reports was devoted to the interactions between different types of nanoparticles and cells[5]. Despite a progress in the application of nanotechnologies in practical biology and medicine, the mechanisms of the possible side effects of nanoparticles are still poorly understood[6, 7].

The gold nanoparticles show great potential as photothermal therapy agents and as imaging agents in connection with its important properties: biocompatibility, high surface reactivity, resistance to oxidation and plasmon resonance[8].

Today some important aspects of the gold nanoparticles effects on cells were revealed that can be used in the treatment of diabetes mellitus and cancer. It is known that both disease characterized by increased production of free radicals due to hyperglycemia and endogenous cancer intoxication, which can be reduced through the use of nanoparticles. Consequently, the gold nanoparticles can use as effective therapeutic agent for the regulation and control oxidative

Saratov Fall Meeting 2014: Optical Technologies in Biophysics and Medicine XVI; Laser Physics and Photonics XVI; and Computational Biophysics, E. A. Genina, V. L. Derbov, K. V. Larin, D. E. Postnov, V. V. Tuchin, Eds., Proc. of SPIE Vol. 9448, 94480U · © 2015 SPIE · CCC code: 1605-7422/15/$18 · doi: 10.1117/12.2179757

stress, which may further significantly improve the quality of life of patients diabetes and cancer. However, the use of new materials in medicine requires detailed examination to clarify the possible side effects, therefore it is necessary to study the effect of gold nanorods in vivo in animals with malignant tumors and diabetes mellitus.

In the literature, very little is known about the influence of gold nanoparticles on the bone marrow in vivo. The aim of the study was to evaluate the influence of gold nanorods on morphological indicators of red bone marrow and peripheral blood of rats with diabetes and transplanted liver tumor after intravenous administration of gold nanorods.

2. METHODOLOGY

In experiments the gold nanorods length of 41±8 nm and a diameter of 10.2±2.0 nm, synthesized in a laboratory of nanobiotechnologies (Institute of Biochemistry and Physiology of Plants and Microorganisms of Russian Academy of Sciences, Saratov, Russia), were used. At synthesis of PEG-coated gold nanorods the following reagents were used: silver nitrate (AgNO3) (>99.9%; Aldrich, 20.913-9), cetyltrimethylammonium bromide (CTAB) (96%; Fluka, USA, № 52370), chloroauric acid (HAuCl4) (>99%; Aldrich, USA), iso-ascorbic acid (AsA) (>99%; Fluka, USA), sodium borohydride (Sigma-Aldrich, USA), hydrochloric acid (OFS, Vekton, Russia), potash (OFS, Reahim, Russia), polyethylene glycol–thiol (PEG-SH) (5000, Nektar, USA), water Milli Q. The first synthesis step was mixed successively 0.1 ml of 1 M CTAB, 25 l of 10 mM HAuCl4 and 100 ul of 10 mM sodium borohydride solution. After the addition of HAuCl4 mixture changes the color from colorless to yellow after the addition of sodium borohydride color immediately changed into pale brown, indicating the formation of gold nanoparticles of 1-3 nm in diameter. Germinal solution was left without stirring, without closing the lid for 30-120 minutes. Next, 2 ml of a 4 mM solution of silver nitrate, 5 ml of a 10 mM solution of HAuCl4, 1 ml of 100 mM solution of ascorbic acid, 1 ml of 1 M hydrochloric acid, 1 ml of gold nuclei were added successively to 100 ml of 0.1 M solution of CTAB. The resulting suspension was heated at 28°C for 24 hours. As a result, a suspension of gold nanorods. CTAB was coated with gold concentration of about 80 ug/ml and an optical density of 3.5-4.0 at 1 cm cuvette at wavelength of the plasmon resonance. The resulting suspension was centrifuged gold nanorods during 1 hour at 14,000 g. The pellet containing the nanoparticles was dissolved in the same volume of water. 1 ml of a 0.2 M solution of potassium carbonate and 1 ml of 1 mM solution of PEG-SH was added to 100 ml of the nanoparticle suspension. The suspension is kept for 12 hours at 28^0C and centrifuged for 1 hour at 14,000 g. The precipitate was dissolved in 100 ml of water. The procedure of centrifugation, redissolution was repeated 5 times, after which the solution was concentrated to a gold concentration 400 ug/ml. Geometrical parameters nanorods were determined by transmission electron microscopy (TEM) images obtained by electron microscope Libra-20 (Carl Zeiss, Germany).

The experimental study was performed at Center of Collective Use of Saratov State Medical University n.a. V.I. Razumovsky (Saratov, Russia) on 48 healthy mature albino male rats weighing 180-220 g. In developing the model of experimental study we relied on the guidance «International Guiding principles for Biomedical Research Involving Animals»[9].

Animals were randomly divided into 4 groups of 12 animals per group: the first experimental group with alloxan diabetes, the second experimental group with transplanted liver cancer, the third experimental group with alloxan diabetes and transplanted liver cancer and the control group. Prior some medical procedure or treatment, the rats were anaesthetized with Zoletil 50 (Virbac, France).

The experimental diabetes induced by intraperitoneal injection of a single dose of alloxan monohydrate – 100 ug/ kg body weight. After administration of alloxan the determination of blood glucose level was carried by Accu-Chek Performa Roche (Switzerland). On the 15th day 6 rats were removed from the experiment by decapitation.

The experimental model of rat liver cancer was reproduced by transplantation of tumor cells suspension of liver cancer (cholangiocarcinoma line PC-1), obtained from the bank of tumor strains of Russian Cancer Research Center n.a. N.N. Blokhin. On the 30th day after transplantation 6 rats were removed from the experiment by decapitation.

In the third group on the 15th day after alloxan injection the liver tumor was transplanted. The modeling diabetes and transplantation tumor carried out similar to methods described above. On the 30th day after transplantation 6 rats were removed from the experiment by decapitation.

All remaining in the experiment rats were injected intravenously gold nanoparticles in amount 2 ml with gold concentration 400 ug/ml. 24 hours after injection all animals were deduced from experiment and blood and bone marrow samples were collected. The bone marrow were taken by the standard procedure of the femur. The smears stained with May-Grunwald for the morphological study. The analysis of bone marrow smears performed under high magnification (x1000) with differential count of 500 cells. The blood analysis was conducted on hematological analyzer PCE-90Vet (USA). The leukogram analysis was conducted by counting of 200 leukocytes in blood smears prepared by the standard procedure. Morphometric analysis was performed using digital image analysis system «Medical Microvizor» μVizo-101 (LOMO, Russia). «SPSS 17.0» program was used for the statistical analysis of the study results with calculation of the arithmetic mean (M), the average error (m) and Student's t test. The results were considered significant at $p < 0,05$.

3. RESULTS

The study of rat blood parameters of all experimental groups allowed to establish a significant decrease in the number of erythrocytes and hemoglobin compared with the control group (Table 1.).

In animals with alloxan diabetes the significant elevation of serum glucose was observed: through 15 days – up to 18.39±3.49 mmol/l which far exceeded the control values. The animals showed clinical manifestations of diabetes - polydipsia, polyuria. The decrease of erythrocyte number and hemoglobin amount was noted in animals with diabetes compared with the control group ($p<0.05$).

At study in the third group of animals with alloxan diabetes and transplanted liver tumor we revealed that the tumor transplantation changes progress of experimental diabetes. 10 days after tumor transplantation blood glucose level returned to normal (to 5.7±0.7 mmol/l). The reducing of tumor growth was noted in third group of animals. The tumor weight 17.6±2.3 g accounted in group with diabetes, in comparison group – 26.9±3.1 g 30 days after transplantation).The marked similar changes of blood indicators were revealed in animal groups with transplanted liver tumor and combined pathology, which may be a compensatory response to hypoxia in the tissues.

The intravenous administration of gold nanorods caused the decrease of hemoglobin and hematocrit indexes in animals of control group and this effect was less pronounced in the animals of experimental groups.

Table 1. Blood indicators at intravenous administration of gold nanorods (GN) in animals of experimental groups

Animal group	Erythrocytes (10^{12}/l)	Hemoglobin (g/l)	Hematocrit (%)	Leucocytes (10^9/l)	Platelets (10^9/l)
Control	7,85±0.51	151,5±13,5	37,7±1,8	9,7±1,6	510±51
Control + GN	6,72±0.86	132,5±13,5	32,3±2,1*	6,95±1,75*	201±28*
Diabetes	6,8±0.1*	133±5,1*	32,5±1,3*	9,75±0,05	517±57
Diabetes + GN	7,1±0,13	139±6,2	33,85±1,65	6,15±1,75*	393±38*
Liver tumor	6,46±0,5*	131±4,4*	33,55±1,75*	7,55±2,75*	789,5±61*
Liver tumor + GN	6,205±0,55	122,5±5,6	32,7±2,55	11,05±1,55	823,5±79
Diabetes and tumor	6,27±0,51*	125±8,3*	32,25±3,2*	6±0,2*	832,5±75*
Diabetes+ tumor+GN	5,84±0,4	114,5±5,7	28,85±1,35	7,95±0,35	778±74

Note: asterisk (*) are distinct from the corresponding values of the control group significantly at $p < 0,05$.

The number of platelets was increased in group with transplanted tumor and in group with combined pathology - diabetes and transplanted tumors. The intravenous administration of gold nanorods caused the decrease of platelets in animals of control group and group with alloxan diabetes.

The significant decrease of leukocyte amount was noted in blood in rats with transplanted tumors and in rats with combined pathology - alloxan diabetes and transplanted tumors. After intravenous administration of gold nanorods the decrease of leukocytes was observed in blood of animals of control group and group with diabetes. In groups with transplanted liver tumors and combined pathology we revealed the increasing of leucocyte number after intravenous administration of gold nanorods.

The leukogram (the percentage of the various forms of white blood cells) was frequently used for assessing the adaptive reactions. After administration of gold nanoparticle the reduction of leukocytes was mainly due to neutrophil granulocytes and monocytes decreasing (Table 2.). Probably the reduction of circulating neutrophils on a background of diabetes is mainly due to the toxic influence of alloxan.

Table 2. Leukogram indicators at intravenous administration of gold nanorods (GN) in animals of experimental groups

Animal group	Eosinophyls (%)	Stab (%)	Segments (%)	Lymphocytes (%)	Monocytes (5)
Control	2±0.5	1,5±0,5	58±3	36,5±1,2	9,5±0,5
Control + GN	2,5±0.5	2±0,5	56,5±2,75	33±1,15	9±0,8
Diabetes	2,5±0.5	1,5±0,2	55,5±0,5	31±1,25	9,5±0,8
Diabetes + GN	1,5±0,3	2±0,5	58±1,75	30±2,75	8,5±1
Liver tumor	1±0,2	3±0,2	53±2,75	37±1,8	6±0,6*
Liver tumor + GN	2±0,2	3±0,3	47±0,5	41±4,05	7±1,1
Diabetes and tumor	1,5±0,14	2±0,2	49±0,5*	41,5±2,2	6,5±0,75*
Diabetes+ tumor+GN	1,5±0,12	2,5±0,2	47±0,35	42,5±1,65	6,5±,75

Note: asterisk (*) are distinct from the corresponding values of the control group significantly at $p < 0,05$.

The study of parameters of bone marrow was revealed the increase of mature forms of leukocytes in animals with alloxan diabetes and transplanted tumors compared with the control group, indicating the activation of myeloid germ (Table 3).

After intravenous administration of gold nanoparticles the decrease of the number of mature cellular elements of the myeloid germ in bone marrow - stab neutrophils and segmented leukocyte was marked in group with diabetes and transplanted tumors. The slight increase of immature elements of mielocytic germ - metamyelocytes was revealed in control group, in group with alloxan diabetes and transplanted liver tumors, indicating the activation of leukocyte germ in these groups of animals.

Table 3. The changes of myeloid cellular forms (%) in bone marrow of rats (M ± m)

Animal group	Myeloblasts	Promyel.	Myelocytes	Metamyel.	Stab	Segments	Eosinoph.	Lymphocyt.
Control	1±0,1	2±0,2	1,5±0,17	0,5±0,07	8±0,8	40,75±4,1	3±0,3	15,75±1,5
Control + GN	1,25±0,2	1,75±0,2	1±0,1	3±0,3*	7,25±0,7	38,5±4,5	4±0,4	15±1,5
Diabetes	0,75±0,08	0,75±0,08*	1,25±0,12	2±0,2*	11±1,3	43,75±4,2	4,25±0,5	13,75±1.7
Diabetes + GN	1,25±0,12	1,5±0,17	1,5±0,2	3,75±0,4	5,75±0,6	38,5±0,39	4±0,4	14,25±1,4
Liver tumor	2,25±0,18	2,25±0,2	1,25±0,12	3,25±0,4*	6,75±0,7	39,5±0,4	1,25±0,12	14±1,5
Tumor + GN	2±0,21	2,25±0,21	1,25±0,13	3,75±0,5	4,75±0,5	36±0,3	5,75±0,6	12,25±1.2
Diabetes and tumor	1,25±0,1	1,75±0,2	0,25±0,02*	7,25±0,8*	7,25±3,8	37,5±0,4	7±0,7*	14,5±1,4
Diabetes+ tumor+GN	1,5±0,2	2±0,2	2±0,2	2,5±0,2	7,5±0,8	47±0,5	3,5±0,4	12,5±1,3

Note: asterisk (*) are distinct from the corresponding values of the control group significantly at $p < 0,05$.

The increasing of erythroblasts and pronormoblasts were noted in animals with transplanted liver tumors and combined pathology indicating the stimulation of erythroid germ of bone marrow (Table 4). In animal groups with alloxan diabetes the decreasing of polychromatic normoblasts were noted ($p < 0,05$).

After intravenous administration of gold nanoparticles the changes of erythroid cellular forms in bone marrow were revealed in control group of animals and in group with liver tumors and combined pathology. The decreasing of polychromatic normoblasts and increasing of oxyphilic normoblasts were noted which probably indicates the increasing of regenerative activity of erythroid hematopoiesis.

Table 4. The changes of erythroid cellular forms (%) in bone marrow of rats (M ± m)

Animal group	Erythroblasts	Pronormoblasts	Bas.normoblasts	Polychr.normoblasts	Oxiphylic normoblasts
Control	2±0,2	2,5±0,2	4±0,4	12,75±1,2	4,5±0,4
Control + GN	2,25±0,3	4,25±0,4	4,25±0,42	7,25±0,8*	8,25±0,8*
Diabetes	1,5±0,12	2,75±0,27	4±0,4	8±0,8*	4,5±0,4
Diabetes + GN	2,5±0,21	3,75±0,37	4,75±0,4	12±1,2	4,25±0,39
Liver tumor	3,75±0,37*	2,75±0,27	3,75±0, 4	10,25±1,1	5,25±0,51
Tumor + GN	1,75±0,17	2,25±0,25	3,75±0,41	10,5±1,0	9,25±0,9*
Diabetes and tumor	3±0,1*	3,5±0,36*	3,25±0,34	10,25±0,8	4,75±0,5
Diabetes+ tumor+GN	1,75±0,2	2,5±0,22	1,75±0,2	5±0,2*	7,25±0,51*

Note: asterisk (*) are distinct from the corresponding values of the control group significantly at $p < 0,05$.

It was found that the administration of gold nanoparticles leads to the changes in the composition of erythroid cells in bone marrow, which may be due to decreasing of red blood cells and hemoglobin in peripheral blood. The administration of nanoparticles leads to a unidirectional change in quantitative and qualitative composition of white blood cells and bone marrow cells of rats. Reducing the number of white blood cells and mature forms of myeloid cells of the bone marrow after nanoparticle administration may be associated with activation of migration function of leukocytes and their transition from the vessels into the tissue after administration of gold nanoparticles, as evidenced by the literature[10,11].

4. CONCLUSION

Thus, in this study we investigated the reaction of the blood and bone marrow cells of rats with intravenous injection of gold nanorods in intact animals and rats with simulate diabetes and transplanted liver tumors. The changes of morphological indicators of blood and bone marrow testify about stimulation of myeloid sprouts of hemopoiesis in bone marrow as a result of decreasing of mature cells in peripheral blood after gold nanoparticle administration. The parameters of homeostasis of blood adequately reflect the state of the major nanoparticle administration. When gold nanoparticles are intravenously administered, they interact with is the blood and its components. Indeed, gold nanoparticles can induce an inflammatory response and change the immune system activity and alter related hematologic factors such as blood cell counts. The duration of the inflammation usually cannot be determined based on leukogram changes alone. It is helpful to embrace the concept of this dynamic situation and consider the bone marrow and tissue environments when interpreting leukogram changes. New data about the influence of gold nanoparticles on the blood and hematopoiesis indicators at the development of alloxan diabetes and transplantation of tumors in rats were obtained.

ACKNOWLEDGMENTS

The study was supported by Government of the Russian Federation (Ministry of Health, state task) and grant №14-13-01167 of Russian Science Foundation.

REFERENCES

[1] Report of a WHO/IDF consultation. [Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia], WHO, 50 (2006).
[2] Müssig, K., Staiger, H., Kantartzis, K., Fritsche, A., Kanz, L., Häring, H.U., "Diabetes, insulin, insulin analogues, and cancer, " Dtsch Med Wochenschr 135(18), 924-929 (2010).
[3] Grote, V.A., Becker, S., Kaaks, R., "Diabetes mellitus type 2 - an independent risk factor for cancer? " Exp. Clin. Endocrinol. Diabetes 118(1), 4-8 (2010).
[4] Boehm, F., [Nanomedical device and systems design nanomedical device and systems design: challenges, possibilities, visions], CRC Press, 787 (2013).
[5] Bellucci, S., [Lecture notes in nanoscale science and technology #7: Nanoparticles and nanodevices in biological applications. The Infn Lectures . Vol I.], Springer, 210 (2008).
[6] Lewinski, N., Colvin, V., Drezek, R., "Cytotoxicity of nanoparticles," Small 4(1): 26–49 (2008).
[7] Gil, P.R., Oberdörster, G., Elder, A., Puntes, V., Parak, W.J., "Correlating physico-chemical with toxicological properties of nanoparticles: The present and the future, " ACS Nano 4(10): 5527–5531 (2010).
[8] Khlebtsov, N.G., Dykman, L.A., "Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies, " Chem Soc Rev 40, 1647-1671 (2011).
[9] International Guiding Principles for Biomedical Research Involving Animals, CIOMS&ICLAS (2012). http://www.cioms.ch/index.php/12-newsflash/227-cioms-and-iclas-release-the-new-international-guiding-principles-for-biomedical-research-involving-animals .
[10] Terentyuk, G.S., Maslyakova, G.N., Suleymanova, L.V., Khlebtsov, B.N., Kogan, B.Ya., Akchurin, G.G., "Circulation and distribution of gold nanoparticles and induced alterations of tissue morphology at intravenous particle delivery, " J. Biophoton. 2(5), 292–302 (2009).
[11] Zhang, X.-D., et.al., "Toxicologic effects of gold nanoparticles in vivo by different administration routes," International Journal of Nanomedicine 5, 771–781 (2010).