The morphological changes in transplanted tumors of rats at plasmonic photothermal therapy

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ABSTRACT

The aim of work was to study the morphological changes in transplanted liver tumors of rats after plasmonic photothermal therapy (PPTT). The gold nanorods functionalized with thiolated polyethylene glycol were injected intravenously to rats with transplanted liver cancer PC-1. A day after injection the tumors were irradiated by the infrared 808-nm diode laser. The withdrawal of the animals from the experiment and sampling of tumor tissue for morphological study were performed 24 hours after the laser exposure. The standard histological and immunohistochemical staining with antibodies to proliferation marker Ki-67 and apoptosis marker BAX were used for morphological study of transplanted tumors. The plasmonic photothermal therapy had pronounced damaging effect n rats with transplanted liver tumors expressed in degenerative and necrotic changes in the tumor cells. The decreasing the proliferation marker Ki-67 and the increasing expression of the apoptosis marker BAX were observed in tumor cells after PPTT.

Keywords: plasmonic photothermal therapy, gold nanorods, transplanted liver tumors, morphological changes

INTRODUCTION

At present, the creation and development of new therapeutic technologies based on the use of nanoparticles for cancer treatment is an urgent problem of medicine.

Laser thermal therapy is commonly used in cancer treatment. The major limitation of such method of therapy is associated with low spatial selectivity of heat affecting both tumor and the surrounding healthy tissue[1]. This restriction may be excluded by with using plasmon-resonant gold nanoparticles as photothermal sensitizers[2]. The considerable amount of studies was focused on the application of various gold nanoparticles for plasmonic photothermal therapy (PPTT).

The unique optical properties and low toxicity of gold nanoparticles make them promising therapeutic agents for cancer treatment. Currently, the use of different gold nanoparticles: nanoshells, nanorods, cages, and other nanocubes proposed for PPTT by several research groups[3-6]. Particularly gold nanorods, due to their structural properties, shows great potential in the fields of photothermal therapy for the treatment of cancer[7].Control of the ratio of the effective absorption and scattering particles, as well as spectral tuning the plasmon resonance of the individual particles in the desired wavelength is achieved by synthesis of nanostructures with different parameters[8,9].

For practical applications, it is preferable to use thermal sensitizers that absorb light in the infrared region (700-1000 nm), where the absorption of tissues themselves is minimal, in so-called therapeutic window transparency of tissues[10]. n addition,

Saratov Fall Meeting 2015: Third Annual Symposium Optics and Biophotonics; Seventh Finnish-Russian Photonics and Laser Symposium (PALS), edited by E. A. Genina, V. L. Derbov, D. E. Postnov, A. B. Pravdin, K. V. Larin, I. V. Meglinski, V. V. Tuchin, Proc. of SPIE Vol. 9917, 991708 · © 2016 SPIE · CCC code: 1605-7422/16/$18 · doi: 10.1117/12.2229873

Selective accumulation of nanoparticles in the biological target can be achieved either by passive or active delivery.

Many tumors have fenestrated vasculature and poor lymphatic drainage, resulting in an enhanced permeability and retention (EPR) effect[11]. Additional surface modification of gold nanoparticles with polyethylene glycol (PEG) prevents agglomeration and improves the stability under conditions of circulation in the circulation[12].

The efficiency of PPTT depends on the right timing and characteristics of the laser hyperthermia after injection of nanoparticles after nanoparticle administration, i.e. when maximum accumulation of nanoparticles in the tumor is observed[13].

The temperature gradient between the tumor and the surrounding healthy tissue dramatically increases at accumulation of nanoparticles in tumor tissue, which provides local heating of the tumor.

To date, the biodistribution of nanoparticles in internal organs are studied as a method of qualitative assessment: transmission electron microscopy, light microscopy[14], confocal microscopy[15], optical coherence tomography[16], and the methods of quantitative assessment of accumulation of nanoparticles in biological tissues: atomic absorption spectrometry[17], method of neutron activation analysis[18]. Toxicity of gold nanoparticles and other nanomaterials directly correlates to accumulation in internal organs, especially in organs of reticuloendothelial system, such as liver and spleen[19]. Passive accumulation of gold nanoparticles nanomaterials can be changed by modifying shape, size, aspect ratio and degree of PEGylation[20].

Despite numerous studies focused on the biomedical application of gold nanoparticles in PPTT, the choice of the most efficient and gentle methods of PPTT was an actual problem of experimental studies.

The aim of this study was to evaluate the tumor and normal tissue distribution of gold nanorods after single and double intravenous administration and to investigate the morphological changes in rat transplanted liver tumors after intravenous injection of gold nanorods and plasmonic photothermal therapy.

METHODOLOGY

Thirty male outbred albino rats with transplanted liver cancer PC-1 were used in the experiment. 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. Suspension was implanted subcutaneously in rats. The experiments were conducted accordingly the guidance "International Guiding principles for Biomedical Research Involving Animals"[21].

When the tumor reached a diameter of 3.0 ± 0.3 cm^3 the animals were randomly divided into three groups (10 rats in group): group 1 - without exposure, group 2 - with single gold nanorods injection and PPTT, group 3 - with double injection of gold nanorods and PPTT. The gold nanorods were synthesized in the Laboratory of Nanobiotechnology (Institute of Biochemistry and Physiology of Plants and Microorganisms of Russian Academy of Sciences, Saratov, Russia)) as described previously[22]. To prevent nanoparticles aggregation in biological tissue and enhance biocompatibility the nanoparticles were functionalized with thiolated polyethylene glycol (MW=5000, Nektar, USA) by previously established method[23]. Geometrical parameters of gold nanorods were determined from analysis of transmission electron microscopy (TEM) images (Libra-120, Carl Zeiss, Germany), which was conducted in Centre of Collective Use of Institute of Biochemistry and Physiology of Plants and Microorganisms RAS. Size of the nanorods was 41±8 nm (length) and 10±2 nm (diameter), and concentration of the nanorods in the suspension was 400 μg/ml, which correspond to optical density of 20 at 808 nm. The gold nanorods in a volume of 1 ml were injected intravenously singly and doubly once a day.

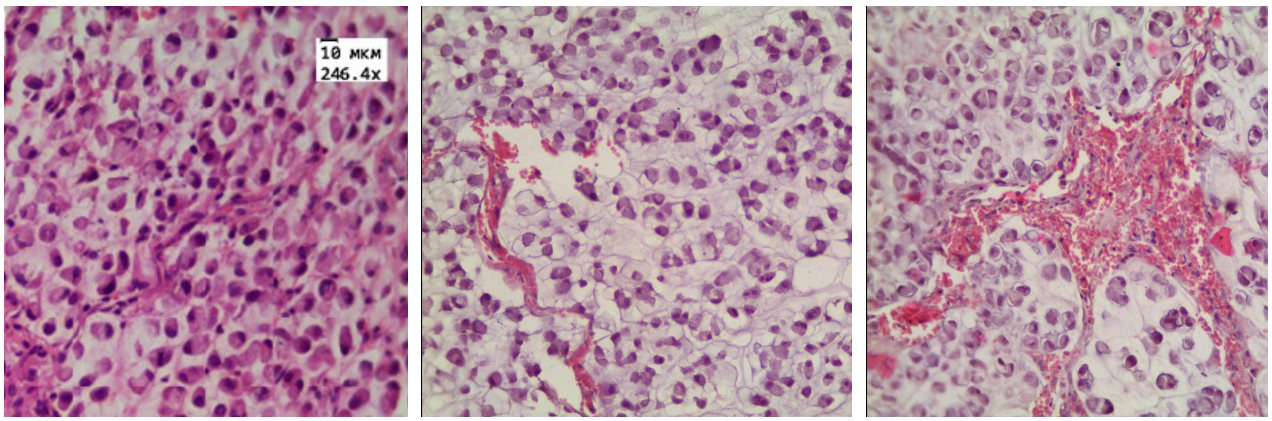
After one day after injection the tumors were irradiated by the infrared 808-nm diode laser LS-2-N-808-10000 (Laser Systems, Ltd., St.-Petersburg, Russia) during 15 min at power density 2.3 W/cm^2. Temperature control of the tumor heating was provided by IR imager IRI4010 (IRYSYS, UK). Prior medical procedure or treatment, the rats were anaesthetized with Zoletil 50 (Virbac, France) in dose of 0.05 mg/kg. The withdrawal of the animals from the experiment and sampling of tumor tissue for morphological study were performed 24 hours after the laser exposure. The standard histological and immunohistochemical staining with antibodies to proliferation marker Ki-67 and apoptosis marker BAX were used for morphological study of transplanted tumors. The determining of gold concentration was conducted in the 1 g of tumor tissue by atomic absorption spectroscopy on spectrophotometer Dual Atomizer Zeeman AA iCE 3500 (Thermo Scientific Inc., USA).

RESULTS AND DISCUSSION

In the first group of rats the transplanted tumors had a lobed structure; segments were separated by thin layers of connective tissue. Tumor cells had oval-rounded shape with eccentrically located nuclei. A significant portion of cytoplasm was occupied by large vacuoles containing mucus. There were clusters of mucous masses in the intercellular spaces (Fig.1, A).

In second group of the rats (single nanoparticle injection and laser hyperthermia) the tumor temperature increased from 35°C up to 42°C. At atomic absorption spectroscopy the gold accumulation in the tumor tissue was insignificant (0.142 ±0.02μg/g) and the temperature increasing was due to only laser hyperthermic influence. The tumor in the group kept lobed structure. There were small foci of necrosis, which take 20-30% of slice area, the tumor cells with necrotibiotic changes were noted. The single mitosis was identified. The vessels were full-blooded, there was thickening of the connective tissue septa and infiltration of leukocytes (Fig.1, B).

In third group of the rats (double nanoparticle injection and laser hyperthermia) we observed the increasing of tumor temperature (up to 46°C) at PPTT. The gold content in the tumor tissue increased almost 9 times (up to 1,236±0.01 μg/g) compared to group with single injection. The more pronounced necrotic changes were revealed in the tumor tissue after PPTT, tumor necrosis occupied up to 30-50% of slice area. The vessels were full-blooded. The tumor cells with necrotibiotic changes were presented in subcapsular zone, (Fig.1, C).

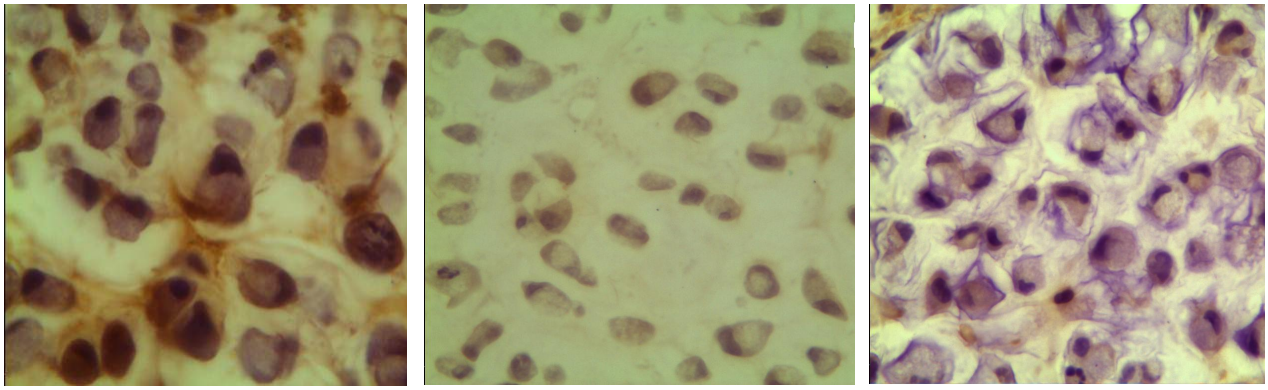


A) B) C)

Fig.1. A) Liver cancer in group without exposure B) Liver cancer after a single intravenous injection of gold nanorods and photothermal therapy B) Liver cancer after double intravenous injection of gold nanorods and photothermal therapy. Staining with hematoxilin and eosin. X246.4.

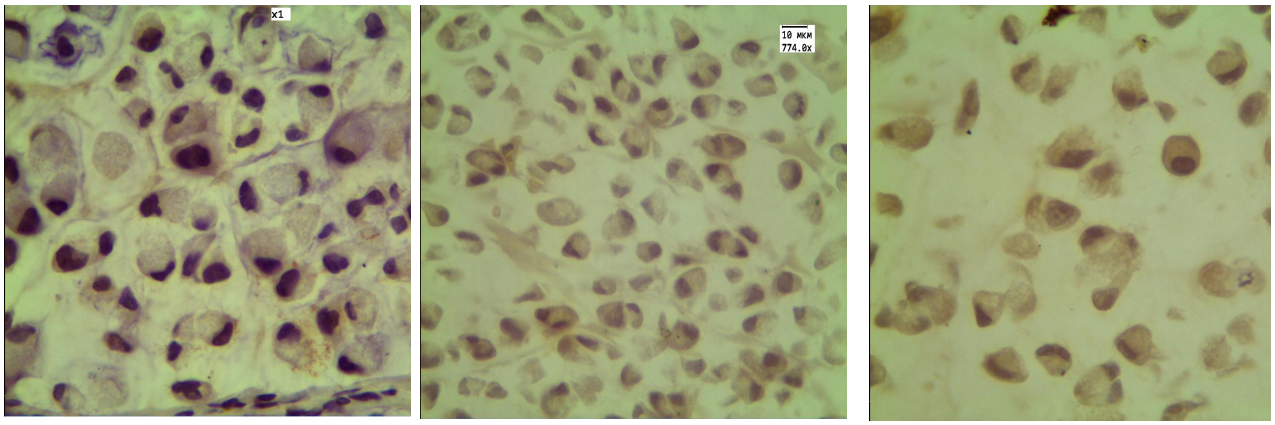
To clarify the impact of photothermal therapy we conducted the immunohistochemical studies of markers of proliferation and apoptosis in tumor tissue. In the first group of rats the transplanted tumors. In the first group of rats the pronounced expression of proliferation marker Ki-67 protein marked in 70% of tumor cells (Fig 2, A). The single intravenous injection of gold nanoparticles and PPTT treatment led to decreasing of Ki-67 expression up to 40-50% of tumor cells (Fig.2., B). The double intravenous injection of gold nanoparticles and PPTT treatment caused more pronounced decreasing of Ki-67 expression up to 40-50% of tumor cells (Fig.2., C).

In the first group of rats the weak expression of apoptosis marker BAX was noted in 20-30% of the tumor cell (Fig.3, A). After single injection of gold nanorods and laser hyperthermia, the weak expression of BAX was revealed in 40-50% of the tumor cells (Fig.3, B).. After double injection of gold nanorods and laser hyperthermia, more marked expression of BAX was revealed in 40-50% of the tumor cells, and a moderate expression was noted in 30% of tumor cells (Fig.3,C). Thus, the decreasing of the proliferation marker Ki-67 expression and increasing of the apoptosis marker BAX expression were observed in tumor cells after PPTT.



A) B) C)

Fig.2 A) Liver cancer in group without exposure B) Liver cancer after a single intravenous injection of gold nanorods and photothermal therapy B) Liver cancer after double intravenous injection of gold nanorods and photothermal therapy. Immunohistochemical staining with antibodies to Ki-67. (×774).



A) B) C)

Fig.3 A) Liver cancer in group without exposure B) Liver cancer after a single intravenous injection of gold nanorods and photothermal therapy B) Liver cancer after double intravenous injection of gold nanorods and photothermal therapy. Immunohistochemical staining with antibodies to BAX. (×774).

In our previous work, intratumorally administered gold nanorods coupled with laser plasmon therapy efficiently impacted the transplanted liver tumors[24]. Herein, we investigate the influence of single and double injections on gold accumulation in tumor tissue and effectiveness of PPTT at such experimental design. The interval of dose repetition (every 24 hours) as well as the time regimen of laser plasmon exposure (24 hours after injection) was developed accordingly our previous work[17] and work of J. Wang et al[13]. The use of GNRs coupled with near IR laser plasmonic therapy successfully destroyed subcutaneously transplanted liver tumor PC-1, caused pronounced necrotic changes in tumor tissue and necrobiotic changes in surviving tumor cells.

CONCLUSION

The experiments showed that the double injection of gold nanorods and laser hyperthermia on transplanted liver tumor in laboratory animals has more pronounced damaging effect expressed in necrotic and degenerative changes in the tumor

cells. The decreasing the proliferation marker Ki-67 and the increasing expression of the apoptosis marker BAX were observed after PPTT.

ACKNOWLEDGEMENTS

The work was supported by grant No. 14-13-01167 from the Russian Science Foundation. The experimental work done by NAN was supported by the Russian President's Scholarship SP-523.2016.4 (2016-2018.).

REFERENCES

[1] Habash, R., et al. "Thermal therapy, part 3: Ablation," Critical Rev. Biomed. Eng. 1, 37-121 (2007).

[2] Huang, X., Jain, P.K., El-Sayed, I.H., El-Sayed, M.A., "Plasmonic photothermal therapy (PPTT) using gold nanoparticles," Lasers Med. Sci 23(3), 217-228 (2008).

[3] Kennedy, L.C., Bickford, L.R., Lewinski, N.A., Coughlin, A.J., Hu, Y., Day, E.S., "A new era for cancer treatment: gold-nanoparticle-mediated thermal therapies," Small 7, 169–183 (2011).

[4] Xia, Y., Li, W., Cobley, C.M., Chen, J., Xia, X., Zhang, Q., "Gold nanocages: from synthesis to theranostic applications," Acc Chem Res 44, 914–924 (2011).

[5] Zhang, J., "Biomedical applications of shape-controlled plasmonic nanostructures: a case study of hollow gold nanospheres for photothermal ablation therapy of cancer," J Phys Chem Lett 1, 686–695 (2010).

[6] Huang, X., El-Sayed, I.H., Qian, W., El-Sayed, M.A., "Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods," J Am Chem Soc 128, 2115–2120 (2006).

[7] Choi, W.I., Sahu, A., Kim, Y.H., Tae, G., "Photothermal cancer therapy and imaging based on gold nanorods,"Ann Biomed Eng 40, 534–546 (2011).

[8] Lee, K.S., El-Sayed, M.A., "Dependence of the enhanced optical scattering efficiency relative to that of absorption for gold metal nanorods on aspect ratio, size, end-cap shape, and medium refractive index," J. Phys. Chem B. 43, 20331-20338(2005).

[9] Khlebtsov, B.N. et al., "Optical amplification of photothermal therapy with gold nanoparticles and nanoclusters," Nanotechnology 17, 5167-5179 (2006).

[10] Tuchin, V.V., [Lasers and fiber optics in biomedical research] 2nd ed., M .: FIZMATLIT, 478 (2010).

[11] Maeda, H., Wu, J., Sawa, T., Matsumura, Y., Hori, K., "Tumor vascular permeability and the EPR effect on macromolecular therapeutics: a review," J. Control. Release 65, 271-284 (2000).

[12] Kogan, B., et al., "Pharmacokinetic study of PEGylated plasmon resonant gold nanoparticles in tumor-bearing mice," Tech. Proc. NSTI Nanotechnol Conf. Trade. 2, 65-68 (2008).

[13] Wang, J., Bai, R., Yang, R., Liu, J., Tang, J., Liu, Y., Li, J., Chaic, Z., Chen C., "Size- and surface chemistry-dependent pharmacokinetics and tumor accumulation of engineered gold nanoparticles after intravenous administration," Metallomics 7, 516-524 (2015).

[14] Gobin, A.M., Lee, M.H., Halas, N.J,, James. W.D., Drezek, R.A., West, J.L., "Near-infrared resonant nanoshells for combined optical imaging and photothermal cancer therapy," Nano Letters 7(7), 1929-1934 (2007).

[15] Alvarez-Román, R., Naik, A., Kalia, Y.N., Guy, R.H., Fessi, H., "Skin penetration and distribution of polymeric nanoparticles," J Control Release 99 (1), 53-62 (2004).

[16] Sirotkina, M.A., Shirmanova, M.V., Bugrova, M.L., Elagin, V.V., Agrba, P.D., Kirillin, M.Yu., Kamensky, V.A., Zagaynova, E.V., "Continuous optical coherence tomography monitoring of nanoparticles accumulation in biologicaltissues," Journal of Nanoparticles research. 13 (1), 283-291 (2011).

[17] Terentyuk, G.S., Maslyakova, G.N., Suleymanova, L.V., Khlebtsov, B.N., Kogan, B.Ya., Akchurin, G.G., Shantrocha, A.V., Maksimova, I.L., Khlebtsov, N.G., Tuchin, V.V., "Circulation and distribution of gold nanoparticles and induced alterations of tissue morphology at intravenous particle delivery," Journal of Biophotonics 2 (5), 292-302 (2009).

[18] James, W.D., Hirsch, L.R., West, J.L., O'Neal, P.D., Payne J.D., "Application of INAA to the build-up and clearance of gold nanoshells in clinical studies in mice," Journal of Radioanalytical and Nuclear Chemistry 271(2), 455-459 (2007).

[19] Goodrich, G.P., Bao, L., Gill-Sharp, K., Sang, K.L., Wang, J., "Photothermal therapy in a murine colon cancer model using nearinfrared absorbing gold nanorods," J Biomed Opt 15, 018001 (2010).

[20] Malugin, A., Ghandehari, H., "Cellular uptake and toxicity of gold nanoparticles in prostate cancer cells: a comparative study of rods and spheres," J Appl Toxicol 30, 212-217 (2010).

[21] ,[International Guiding Principles for Biomedical Research Involving Animals], CIOMS&ICLAS, http://www.cioms.ch/index.php/12-newsflash/227-cioms-and-iclas-release-the-new-international-guiding-principles-for-biomedical-research-involving-animals (2012).

[22] Alekseeva, A.V., Bogatyrev, V.A., Khlebtsov, B.N., Melnikov, A.G., Dykman, L.A., Khlebtsov, N.G., "Gold nanorods: Synthesis and optical properties," Colloid Journal 68, 661 (2006).

[23] Khlebtsov, B.N., Tuchina, E.S., Khanadeev, V.A., Panfilova, E.V., Petrov, P.O., Tuchin, V.V., Khlebtsov, N.G., "Enhanced photoinactivation of Staphylococcus aureus with nanocomposites containing plasmonic particles and hematoporphyrin," J. Biophotonics 6, 338 (2013).

[24] Bucharskaya, A.B., Maslyakova, G.N., Afanasyeva, G.A., Terentyuk, G.S., Navolokin, N.A., Zlobina, O.V., Chumakov, D.S., Bashkatov, A.N., Genina, E.A. Khlebtsov, N.G., Khlebtsov, B.N., Tuchin V.V., "The morpho-functional assessment of plasmonic photothermal therapy effects on transplanted liver tumor," Journal of Innovative Optical Health Sciences 8(3),1541004 (2015).