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The inflammation markers in serum of tumor-bearing rats after plasmonic photothermal therapy

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ABSTRACT

We report on plasmonic photothermal therapy of rats with inoculated cholangiocarcinoma through the intratumoral injection of PEG-coated gold nanorods followed by CW laser light irradiation. The length and diameter of gold nanorods were 41±8 nm and 10±2 nm, respectively; the particle mass-volume concentration was 400 μg/mL, which corresponds to the optical density of 20 at the wavelength 808 nm. The tumor-bearing rats were randomly divided into three groups: (1) without any treatment (control); (2) with only laser irradiation of tumor; (3) with intratumoral administration of gold nanorods and laser irradiation of tumors. An hour before laser irradiation, the animals were injected intratumorally with gold nanorod solutions in the amount of 30% of the tumor volume. The infrared 808-nm laser with power density of 2.3 W/cm^2 was used for plasmonic photothermal therapy (PTT). The withdraw of animals from the experiment was performed 24 h after laser exposure. The content of lipid peroxidation products and molecular markers of inflammation (TNF-α, IGF-1, VEGF-C) was determined by ELISA test in serum of rats. The standard histological techniques with hematoxylin and eosin staining were used for morphological examination of tumor tissues. It was revealed that the significant necrotic changes were noted in tumor tissue after plasmonic photothermal therapy, which were accompanied by formation of inflammatory reaction with release of proinflammatory cytokines and lipid peroxidation products into the bloodstream.

Keywords: plasmonic photothermal therapy (PTT), gold nanorods, antitumoral therapy, inflammation markers

INTRODUCTION

At present time, nanotechnology occupies an important place among the innovative methods of diagnostics and treatment in medicine. The search of new effective agents for therapy of malignant neoplasms is one of the promising directions for nanotechnology application.[1]

Gold nanoparticles have extraordinary physicochemical properties: biocompatibility, high reactivity surface, resistance to oxidation, ability to plasmon resonance, which determine their significance for use in biomedical applications.[2-4] A lot of works demonstrate that the range of nanotechnology applications in biomedicine is quite wide, for example, gold nanoparticles can be used to enhance contrast and improve diagnostic sensitivity in OCT,[5,6] for targeted delivery,[7,8] photodynamic[9,10] and photothermal therapy.[11,12]

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The sensitization of tumor tissue by administration of gold nanoparticles is a prospective way of increasing the selectivity of the laser heating.[13] Currently, such technology has been developed and it was named a plasmonic photothermal therapy (PTT).[14] To achieve highly effective photothermal ablation of cancer cells, the following parameters should be optimized: the size and shape of gold nanoparticles, the administration method for sufficient accumulation of nanoparticles in tumors and the mode of laser exposure. Accumulated information about effects of gold nanoparticles on animal organism are essential for the prognosis of therapeutic efficacy and suitability for in vivo application. Earlier experiments on the effect of laser hyperthermia on the internal organs of rats with intertwined tumors with intramural insertion of gold nanorods were described in our work.[15]
Here, we investigate gold nanorod (GNR)-mediated laser-induced photothermal effects on molecular markers of inflammation, cellular interaction and peroxidation indicators in serum of tumor-bearing rats.

MATERIAL AND METHODS

Animal experiments were performed in accordance with recommendation of the University's Animal Ethics Committee and the relevant national agency regulating animal experiments in Centre of Collective Use of Saratov State Medical University. A total of 18 male outbred albino rats weighing 200±20 g were used to study the effect of laser hyperthermia with a single intratumoral injection of GNRs. 0.5 mL of 25% tumor cell suspension of alveolar liver cancer — cholangiocarcinoma PC-1, obtained from the bank of tumor strains of Russian Cancer Research Center n.a. N.N. Blokhin (Moscow, Russia), was implanted subcutaneously in rats. When the tumor reached a diameter of 3± 0.3 cm, the animals were randomly divided into three groups (6 rats in each group): group 1 without any exposure (control group), group 2 with the laser irradiation of the tumor, group 3 with intratumoral administration of gold nanorods and laser irradiation of the tumor.

GNRs were synthesized by previously established method.[16] To prevent nanoparticle aggregation in tissue and enhance biocompatibility, nanoparticles were functionalized with thiolated polyethyleneglycol (Mw =5000, Nektar, USA) by previously established method.[17] Geometrical parameters of GNRs were determined from analysis of transmission electron microscopy (TEM) images (Libra-120, Carl Zeiss, Germany) in Centre of Collective Use of IBPPM RAS. Extinction spectra of samples were measured with a Specord 250 BU spectrophotometer (Analytik, Jena, Germany). Size of the nanorods was 41±8 nm in length and 10±2 nm in diameter and concentration of nanorods in the suspension was 400 μg/mL, which corresponds to optical density of 20 near the plasmon resonance wavelength.

The infrared 808-nm CW laser LS-2-N-808-10000 (Laser Systems, Ltd., St. Petersburg, Russia) with a power density of 2.3 W/cm^2 was used for PTT. An hour before laser irradiation, the animals were injected intratumorally with the solution of gold nanorods in the amount of 30% of the tumor volume. Irradiation was carried out percutaneously within the area of a tumor during 15 min. Temperature control of the tumor heating was provided by IR imager IRI4010 (IRYSYS, UK).

Prior to any medical procedure or treatment, the rats were anaesthetized with Zoletil 50 (Virbac, France) in dose of 0.05 mg/kg. The removal of animal from the experiment was performed 24 h after laser exposure. The tissue samples of internal organs and tumors for morphological studies were collected. The standard histological techniques with hematoxylin and eosin staining were used.

The study of lipid peroxidation activity was carried out using measuring of intermediate products of lipid peroxidation — malondialdehyde (MDA) and lipid hydroperoxide (LHP)—in the blood serum of experimental animals with standard biochemical methods on spectrofluorometer RF-5301 PC (Shimadzu Corp., Japan). MDA was determined by reaction with 2-thiobarbituric acid. The absorbance of samples was measured by spectrophotometric recording at λ = 532 nm, the concentration of MDA was expressed in μM/mL. The method of hydroperoxide determination was based on absorption of ultraviolet radiation by lipid hydroperoxide at the wavelength of 233nm. The results were expressed in relative optical density units per 1mL of plasma.

The method of enzyme immunoassay (ELISA) was used to study molecular markers: a pro-inflammatory marker - tumor necrosis factor TNF-α (Rat TNF-α Platinum Elisa Kit, eBioscience Inc.), growth factors: insulin-like growth factor IGF-1 (Rat / Mouse IGF-1 Elisa Kit, Novozymes Inc.), vasculoendothelial growth factor VEGF-C (Platinum Elisa Kit, eBioscience Inc.). The analysis of data was conducted by using of SSPS-13.0 statistical programs.

RESULTS AND DISCUSSION

The levels of lipid peroxidation products (MDA, LHP) did not change in serum of control animals after single intravenous administration of GNRs (Fig.1, a). No significant changes in tumor necrosis factor (TNF-α), insulin-like growth factor IGF-1 and vasculoendothelial factor were observed in the control group of animals after single intravenous administration of gold nanorods (Table 1). No significant changes were observed in internal organs of control and tumor-bearing animals after single injection of gold nanorods.

The growth of transplanted tumors is accompanied by increase of the lipid peroxidation activity and proinflammatory response. It was revealed that the levels of lipid peroxidation products (MDA, LHP) and TNF-α in serum of tumor-bearing rats were significantly higher than those parameters in the control group (Table 1, Fig. 1, a). The amount of IGF-1 and VEGF-C does not change significantly. The morphological features of transplanted tumors correspond to cholangiocarcinoma from the cells of the bile ducts of the liver (Fig.1,b).

Table 1

ELISA data of molecular markers in serum

Animal groups	TNF-α (pg/ml) Mediana [lower quartile; upper quartile]	IGF– 1(ng/l) Mediana [lower quartile; upper quartile]	VEGF-C (pg/ml) Mediana [lower quartile; upper quartile]
Control	4 [2;6]	1450 [1250;1760]	27 [24;38]
Control group after IV injection of GNRs	5 [3;10]	1040 [658;1290]	25 [19;44]
Liver cancer	21 [14;47]*	940 [504;1330]	27 [16;36]
Liver cancer after IV injection of GNRs	18 [3;61]	751 [569;1270]*	56 [50;61]*
Liver cancer after only laser irradiation	16 [5;47]	926 [458;1180]	21 [14;29]
Liver cancer after PTT	28[3;52]*	1295[904;1470]	26[17;36]

Note: * $p < 0.05$ compared with control group

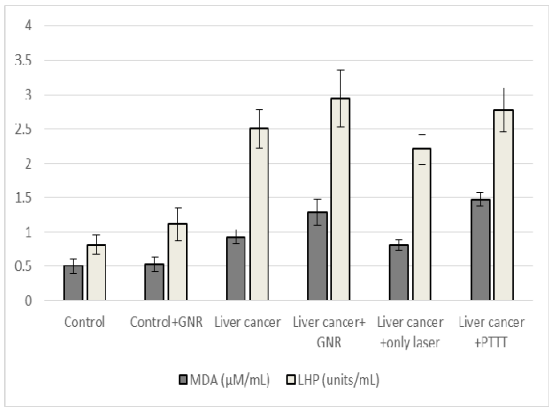
After intravenous administration of gold nanorods the content of MDA and LHP did not change in serum of tumor-bearing rats, the level of IGF-1 slightly decreased, but the level of VEGF-C was significantly increased. It is known that VEGF-C increases vascular permeability as well as the migration and proliferation of endothelial cells[18] and may have the stimulating effect on the processes of lymph-angiogenesis in tumor-bearing rats.[19]

The size, shape, charge, and surface modification of gold nanoparticles (GNPs) largely determine the biodistribution, toxicity, and biocompatibility of gold nanoparticles. In work[20], Zhang et al. have reported that 60 nm-GNPs are neither cytotoxic nor elicit pro-inflammatory responses (IL-6, TNF-α) in murine macrophages despite the cellular uptake of GNPs and localization within intracellular vacuoles were evident. Yen et al.[21] have found that negatively charged GNPs might adsorb serum protein, enter cells via the more complicated endocytotic pathway and upregulate the expressions of proinflammatory genes (IL-1,IL-6,and TNF-α). Khan et al.[22] confirmed the increased expression of pro-inflammatory cytokines in liver after acute exposure to 10 and 50 nm GNPs, but these changes were reversible. Also, in work of Chiodo et al.,[23] it was noted that the expression of IL-6 cytokines and TNF-α increased under the influence of GNPs

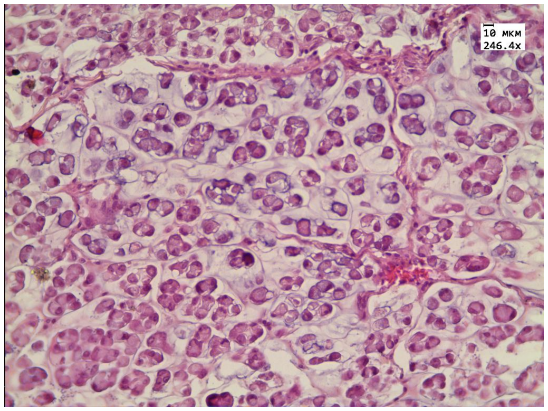
coated with the galactofuranose. These differences in GNPs effects, probably, may be due to the influence of size and coating of nanoparticles.

At only laser exposure, the superficial tumor temperature increased up to 42°C, at morphological examination the necrotic changes in some tumor cells and small foci of necrosis were noted (Fig.1,c). Thickening of the connective tissue septa and focal hemorrhages were observed. MDA and HPL levels were higher than those control indicators, but insignificantly differed from the indicators of tumor-bearing rats without any treatment.

We noted a significant increase in tumor superficial temperature (up to 60°C) at PTT of tumors after intratumoral injection of gold nanorods as nanosensitizers. The pronounced necrotic changes developed in the tumor center, which occupied up to 80–90% of the slice (Fig.1, d). The tumor cells with marked degenerative changes were preserved only in subcapsular tumor zone. The formation of massive necrosis in tumor tissues was accompanied by the development of pro-inflammation reaction and increased lipid peroxidation processes. The excessive accumulation of MDA and HPL and increased TNF-α level were revealed in serum of tumor-bearing rats after PTT exposure compared with group of animals exposed to only laser irradiation. The content of IGF-1 and VEGF-C does not change significantly.



a



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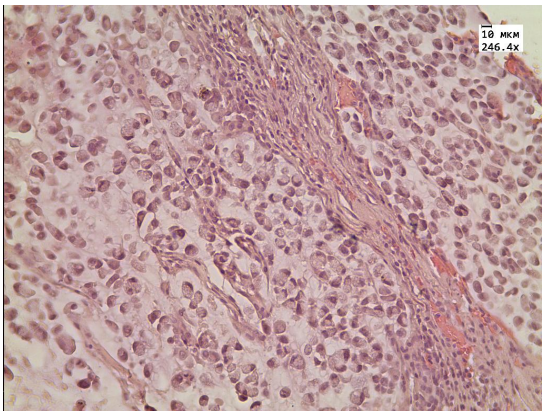

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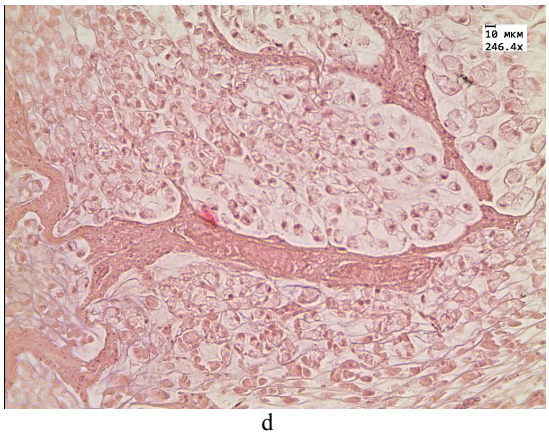

d

Figure1. Indicators of lipid peroxidation in serum of rats (a); liver cancer without any treatment (b); Liver cancer after only laser, the necrotic changes in some tumor cells (c); Liver cancer after PTT. Pronounced necrosis in tumor tissue (d). H&E. x246.4 .

CONCLUSION

Single intravenous administration of PEG-coated gold nanorods did not lead to significant changes in the intensity of lipid peroxidation processes and pro-infalmmation markers in blood serum of intact animals, which allows us torecommend their application as sensitizers for PTT. The pronounced necrotic changes in the tumor tissue after PTT

may be accompanied by the accumulation of intermediate products of lipid peroxidation and the release of pro-inflammatory markers in serum of rats that can be result of membrane damage of tumor cells at PTT.

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REFERENCES

[1] Wang, X., Wang, X., Yang, L., Chen, Z.G., Shin, D.M., "Application of nanotechnology in cancer therapy and imaging," CA Cancer J. Clin. 58, 97–110 (2008).
[2] Dreaden, E.C., Alkilany, A.M., Huang, X., Murphy, C.J., El-Sayed, M.A., "The golden age: Gold nanoparticles for biomedicine," Chemical Society Reviews. 41, 2740–2779(2012).
[3] Dykman, L.A., Khlebtsov, N.G., "Gold nanoparticles in biomedical applications: recent advances and perspectives," Chem Soc Rev. 41, 2256e82 (2012).
[4] Abadeer, N.S., Murphy, C.J., "Recent progress in cancer therapy using gold nanoparticles," J Phys Chem 120, 4691–4716 (2016).
[5] Zagaynova, E.V., Shirmanova, M.V., Kirillin, M.Y., Khlebtsov, B.N., Orlova, A.G., Balalaeva, I.V., Sirotkina, M.A., Bugrova, M.L., Agrba, P.D., and Kamensky, V.A., "Contrasting properties of gold nanoparticles for optical coherence tomography: phantom, in vivo studies and Monte Carlo simulation," Phys. Med. Biol. 53, 49952008 (2008).
[6] Genina, E.A., Terentyuk, G.S., Khlebtsov, B.N., Bashkatov, A.N., and Tuchin, V.V., "Visualisation of distribution of gold nanoparticles in liver tissues ex vivo and in vitro using the method of optical coherence tomography," Quantum Electron. 42, 478 (2012).
[7] Dreaden, E.C., Austin, L.A., Mackey, M.A., El-Sayed, M.A., "Size matters: gold nanoparticles in targeted cancer drug delivery," Ther Deliv. 3(4), 457–478 (2012).
[8] Niikura, K., Iyo, N., Matsuo, Y., Mitomo, H., Ijiro, K., "Sub-100 nm gold nanoparticle vesicles as a drug delivery carrier enabling rapid drug release upon light irradiation," ACS Appl Mater Interfaces. 5, 3900-3907 (2013).
[9] Triesscheijn, M., Baas, P., Schellens, J.H.M., and Stewart, F.A., "Photodynamic therapy in oncology," Oncologist. 11(9), 1034–1044 (2006).
[10] Terentyuk, G., Panfilova, E., Khanadeev, V., Chumakov, D., Genina, E., Bashkatov, A., Tuchin, V., Bucharskaya, A., Maslyakova, G., Khlebtsov, N., Khlebtsov, B., "Gold nanorods with hematoporphyrin-loaded silica shell for dual-modality photodynamic and photothermal treatment of tumors in vivo," Nano Res. 7, 325-337 (2014).
[11] Terentyuk, G.S., Maslyakova, G.N., Suleymanova, L.V., Khlebtsov, N.G., Khlebtsov, B.N., Akchurin, G.G., et al., "Laser-induced tissue hyperthermia mediated by gold nanoparticles: Toward cancer," Journal of Biomedical Optics, 14(2), 021016 (2009).
[12] X. Huang, P. K. Jain, I. H. El-Sayed, M. A. El-Sayed, "Gold nanoparticles and nanorods in medicine: From cancer diagnostics to photothermal therapy," Nanomedicine 2, 681–693 (2007).
[13] 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).
[14] Huang, X., El-Sayed, M.A., "Plasmonic photo-thermal therapy (PPTT)," Alexandria Journal of Medicine. 47, 1–9 (2011).
[15] 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," J. Innov. Opt. Health. Sci. 8, 1541004 (2015).
[16] 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. 68,661–678 (2006).

[17] Khlebtsov, B.N., Tuchina, E.S., Khanadeev, V.A., Panfilova, E.V., Petrov, P.O., Tuchin, V.V., "Enhanced photoinactivation of Staphylococcus aureus with nanocomposites containing plasmon particles and hematoporphyrin," J. Biophotonics. 6(4), 338–351 (2013).
[18] Joukov, V., Sorsa, T., Kumar, V., Jeltsch, M., Claesson-Welsh, L., Cao, Y., Saksela, O., Kalkkinen, N., and Alitalo, K., "Proteolytic processing regulates receptor specificity and activity of VEGF-C," EMBO J. 13, 3898-3911 (1997).
[19] Alitalo, K., Carmeliet, P., "Molecular mechanisms of lymphangiogenesis in health and disease," Cancer Cell. 1, 219-227(2002).
[20] Zhang, Q., Hitchins, V.M., Schrand, A.M., Hussain, S.M., and Goering, P.L. "Uptake of gold nanoparticles in murine macrophage cells without cytotoxicity or production of pro-inflammatory mediators," Nanotoxicology. 5, 284–295 (2011).
[21] Yen, H.J., Hsu, S.H., Tsai, C.L., "Cytotoxicity and immunological response of gold and silver nanoparticles of different sizes," Small. 5(13), 1553–1561 (2009).
[22] Khan, H.A., Abdelhalim, M.A.K., Alhomida, A.S., Al Ayed, M.S., "Transient increase in IL-1β, IL-6 and TNF-α gene expression in rat liver exposed to gold nanoparticles," Genetics and Molecular Research. 12 (4), 5851-5857 (2013).
[23] Chiodo, F., Marradi, M., Park, J., Ram, A.F., Penadés, S., van Die, I., Tefsen, B., "Galactofuranose-coated gold nanoparticles elicit a pro-inflammatory response in human monocyte-derived dendritic cells and are recognized by DC-SIGN,"ACS Chem Biol. 9(2), 383-389 (2014).