

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 in 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 tissue¹. This restriction may be excluded by with using plasmon-resonant gold nanoparticles as photothermal sensitizers². 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 groups³⁻⁶. Particularly gold nanorods, due to their structural properties, shows great potential in the fields of photothermal therapy for the treatment of cancer⁷. 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 tissues¹⁰. In addition,

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) effect¹¹. Additional surface modification of gold nanoparticles with polyethylene glycol (PEG) prevents agglomeration and improves the stability under conditions of circulation in the circulation¹².

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 observed¹³.

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 microscopy¹⁴, confocal microscopy¹⁵, optical coherence tomography¹⁶, and the methods of quantitative assessment of accumulation of nanoparticles in biological tissues: atomic absorption spectrometry¹⁷, method of neutron activation analysis¹⁸. Toxicity of gold nanoparticles and other nanomaterials directly correlates to accumulation in internal organs, especially in organs of reticuloendothelial system, such as liver and spleen¹⁹. Passive accumulation of gold nanoparticles nanomaterials can be changed by modifying shape, size, aspect ratio and degree of PEGylation²⁰.

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 $3.0 \pm 0.3 \text{ cm}^3$ 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 previously²². 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 method²³. 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 $41 \pm 8 \text{ nm}$ (length) and $10 \pm 2 \text{ nm}$ (diameter), and concentration of the nanorods in the suspension was $400 \mu\text{g/ml}$, 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.

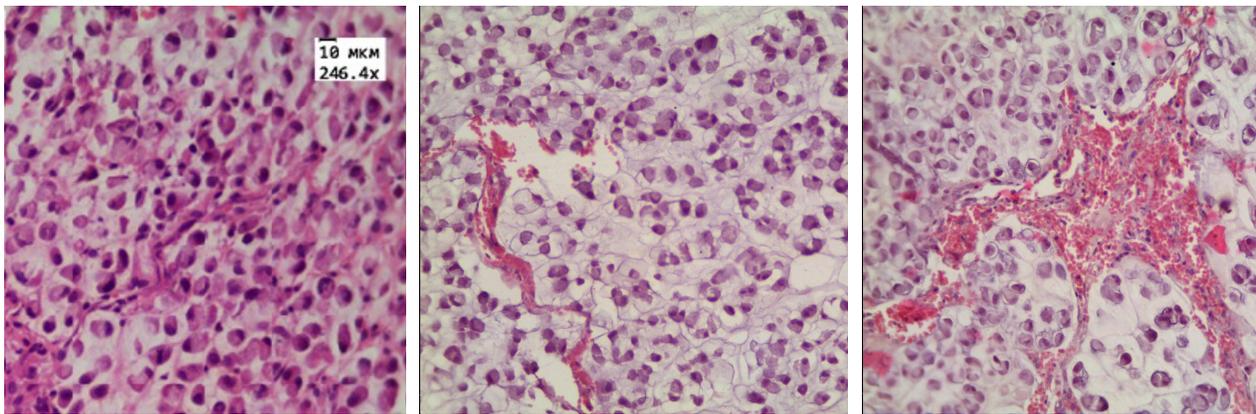
After one day after injection the tumors were irradiated by the infrared 808-nm diode laser LS-2-N-808-10000 (Laser Systems, Ltd., St.-Petersburg, Russia) during 15 min at power density 2.3 W/cm^2 . Temperature control of the tumor heating was provided by IR imager IRI4010 (IRYSYS, UK). Prior medical 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 ($0.142 \pm 0.02 \mu\text{g/g}$) 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 necrotic 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 $1,236 \pm 0.01 \mu\text{g/g}$) 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 necrotic changes were presented in subcapsular zone, (Fig.1, C).



A)

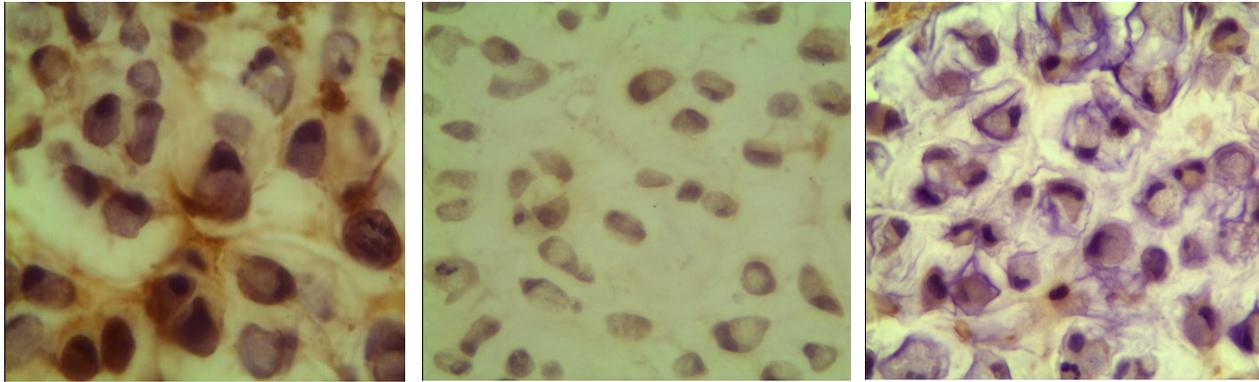
B)

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 hematoxylin 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.

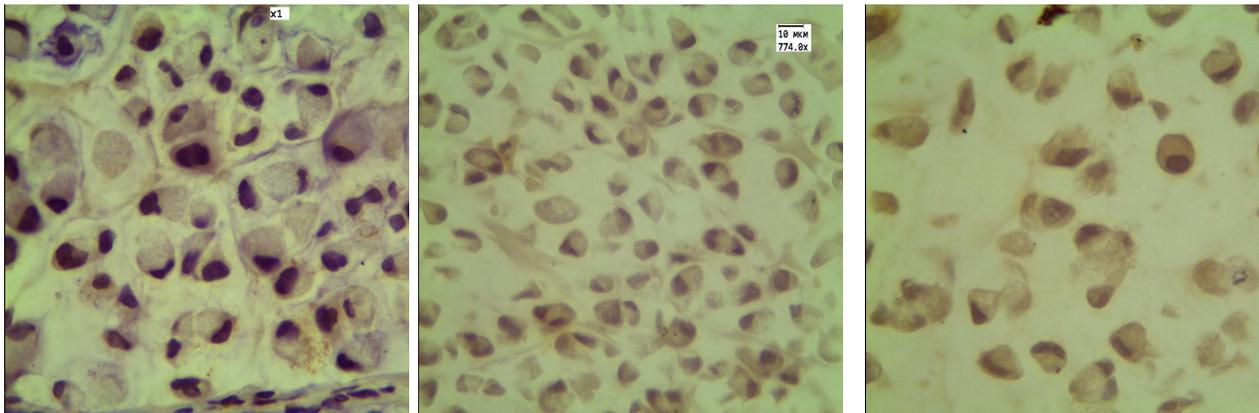


A)

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. ($\times 774$).



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. ($\times 774$).

In our previous work, intratumorally administered gold nanorods coupled with laser plasmon therapy efficiently impacted the transplanted liver tumors²⁴. 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 work¹⁷ and work of J. Wang et al¹³. 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.

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