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Treatment of the *Graffi* Tumor in Hamsters Using Plasmonically Activated Gold Nanoparticles

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Summary: Local application of heat is a well-known concept in therapeutic medicine that has been explored extensively for the treatment of cancer and other conditions. This study has been designed to determine the photothermal properties of plasmonically heated gold nanoparticles (GNPs) in vivo, using experimental animal model - solid myeloid *Graffi* tumor in hamsters. Combining cytochemical, biochemical and histopathological methods we found that combination of GNPs (40 nm and 100 nm) and laser treatment with different characteristics of the laser beam resulted in localized heating and causing local destruction of the tumor tissue, prolonged survival rate and mean survival time of the tumor bearing animals. This study demonstrates that GNPs are a novel class of photothermal agents which cause cell injury and death through conversion of absorbed light to thermal energy.

Keywords: gold nanoparticles, photothermal therapy, *Graffi* tumor

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Introduction

The revolution in cancer therapy has taken place by emerging use of laser light to achieve controlled and confined thermal damage in the tumor tissue. Laser is an optical source that emits photons in a coherent and narrow beam [1]. Noble metal nanoparticles have become very useful as agents for photothermal therapy of their enhanced absorption cross sections, which are four to five orders of magnitude larger than those offered by conventional photoabsorbing dyes. This strong absorption ensures effective laser therapy at relatively lower energies rendering the therapy method minimally

invasive. Irradiation with short laser pulses has been shown to lead to rapid heating of the particles and vaporization of thin layer of fluid surrounding each particle, producing microscopic explosions and bubble formation [3-5, 7-9]. Clusters formed by the assembly of gold nanoparticles enhance the bubble formation, causing more efficient cancer cell killing [8].

Aim

The aim of the present study is to elucidate the effects of local application of gold nanoparticles in combination with laser beam irradiation on parameters of the tumor growth and histopathological evaluation of the tumor tissue damage.

Materials and Methods

Golden Syrian hamsters, 2-4 months old, weighing approximately 100 g were purchased from a breeding base Oncology Center, Sofia. The animals were divided into experimental groups and were kept under standard conditions in individual plastic cages with free access to food and water. All studies were performed in accordance with the Guide for Care and Use of Laboratory Animals, as proposed by the Committee on Care Laboratory Animal Resources, Commission on Life Sciences and National Research Council. An experimental *Graffi* myeloid tumor was created and maintained monthly in vivo by subcutaneous transplantation of live tumor cells by method described by Toskova et al., 2008 [6]. Spontaneous regression in this experimental tumor model was not observed. The tumors were irradiated using Nd-YAG laser at λ = 532 nm, pulse duration τ p= 15 ns and repetition rate 1 Hz. Gold nanoparticles (GNP) with diameters of 40 nm and 100 nm (BBInternational, Cardiff, UK) were used as colloid solutions without surfactants, stabilizers or enhancers. Changes in tumor volume and mean survival time (MST) of tumor-bearing hamsters after combination laser/GNP therapy were followed. Untreated tumor-bearing and healthy animals were used as controls. Samples of tumor tissue were selected for histopathological studies. They were obtained from animals from each experimental group and were processed and stained with haematoxylin-eosin according to the standard histological technique. At the 72nd hour after treatment, experimental tumor bearing animals from different groups were euthanized. The solid tumors were dissected and selected parts were immediately fixed for 48 hours in 10% phosphate buffered formalin pH 7.2 (end formalin concentration was 3,8 – 4%), included in paraffin and cut in sections of 4 μm. Representative histological sections were stained with hematoxylin-eosin.

Some experiments were carried out aimed to clarify whether inhibition of proliferation of *Graffi* tumor cells takes place through apoptosis. For this purpose primary culture of *Graffi* tumor cells were cultured for 4 h on coverslips, then colloid gold was added to each sample in end concentration 10 μg/mL and cells were cultured for 24 hours to ensure the passive transport of the GNPs into the tumor cells. Cells were irradiated with Nd-Yag laser system with parameters of the laser beam depending on the requirements of the experiment. After two hours fluorescent analysis was performed. AO stains both viable and dead cells emitting strong green fluorescence, as a result of intercalation between the bases of double-stranded DNA and red-orange fluorescence after binding to single-stranded RNA [2]. In contrast, PI is a fluorochrome which does not stain viable cells with intact cell membrane. It stains the dead and late apoptotic cells with altered cell membrane permeability.

Results and discussion

Morphological changes in *Graffi* tumor cells treated with gold nanoparticles and irradiated with laser beam with different energies observed with fluorescent microscopy are shown in Fig. 1. The nucleus of the untreated *Graffi* cells showed homogenous fluorescence with no signs of segmentation and fragmentation. Cells treated with gold nanoparticles and irradiated exhibited different signs of early and late apoptosis. The obtained results support the claim that the combination of gold nanoparticles (40 nm and 100 nm) and laser irradiation induces death of *Graffi* tumor cells through apoptosis.

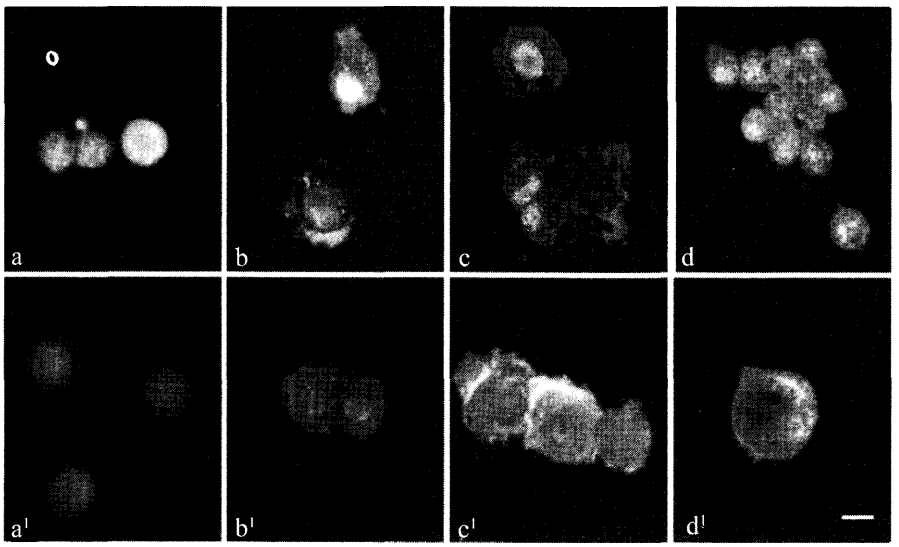

Fig. 1. Fluorescent micrography of *Graffi* tumor cells, stained with acridine orange and ethidium bromide. Bar = 20 μm

The combination of GNP and laser therapy on hamsters with *Graffi* tumor showed temporary positive effect on the metric parameters of the tumor growth, expressed in reduction of tumor volume and prolonged mean survival time (data not shown).

The results observed in native scanned histological preparations showed that at the 72nd hour after treatment (Fig. 2) a narrow zone of necrotic effect in the tumor tissue. This zone lays on the axis of action of the laser beam and is well pronounced when the tumor was treated with 40 nm gold nanoparticles, while in tumors treated with 100 nm nanoparticles this zone of destruction is much wider (Fig, 2A). At the 7th day after the treatment the zone of necrotic alterations in the tumor tissue is unclear due to the lateral growth of the tumor tissue, remained unaffected from the photodynamic therapy (Fig. 2B). In the cases with small tumor formations the neoplastic tissue was totally destructed (data not shown).

The treatment of *Graffi* tumor bearing animals with gold nanoparticles and laser irradiation induces pathomorphological changes in the zone of treatment, shown in Figure 3. These changes detected by pathohistological methods could be classified in the following zones:

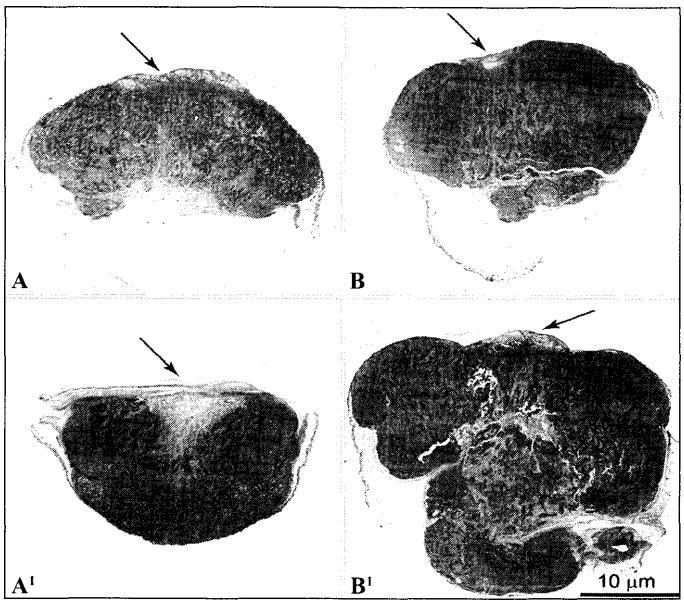

Fig. 2. Solid *Graffi* tumor in hamsters at 3rd (A, A¹) and 7th (B, B¹) day after combined treatment with nanoparticles and laser. (A, B) – Zone of tumoricide effect on the neoplastic tissue (arrow) after treatment with gold nanoparticles (40 nm) and laser (80 mJ/cm²) at 3rd and 7th day respectively; (A¹, B¹) – Zone of tumoricide effect on the neoplastic tissue (arrow) after treatment with nanoparticles (100 nm) and laser (80 mJ/cm²) at 3rd and 7th day respectively. Scanned native histological preparations

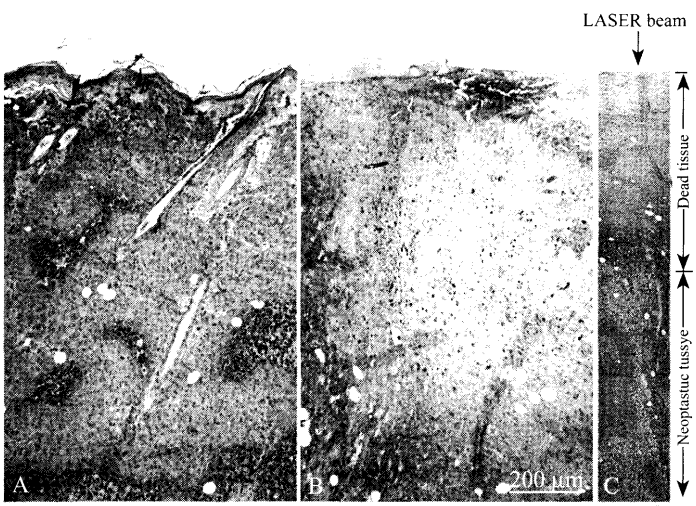

Fig. 3. Solid myeloid *Graffi* tumor in hamster, inoculated with gold nanoparticles (40 nm). (A) – untreated surface with intact structure; (B) – totally destructed skin and neoplastic tissue in laser treated tumor; (C) from top to bottom – skin, dead tumor tissue and viable neoplastic tissue. Hematoxylin- eosin

a. Superficial zone of total necrotic tissue (SZTNT) – necrotic tumor cells as cell debris with nuclear fragments or entirely lytic cells with pale nuclei (Fig. 4a);
b. Middle superficial zone of necrotic tissue (MSZNT) – lytic cells and basophilic agglomerations. (Fig. 4b);
c. Middle deep zone of necrotic tissue (MDZNT) – tumor necrotic tissue, hemorrhages (Hrrg) and inflammatory mononuclear cells (Mo) (Fig. 4c);
d. Zone of deep necrotic tissue (ZDNT) – necrotic tissue with inflammatory mononuclear cells (Mo) (Fig. 4d);
e. Zone of deep neoplastic tissue (ZDNeoT) and Zone of lateral neoplastic tissue (ZLNeoT) – neoplastic tissue with the specific characteristics of myeloid *Graffi* tumor in hamsters (Fig. 4e).

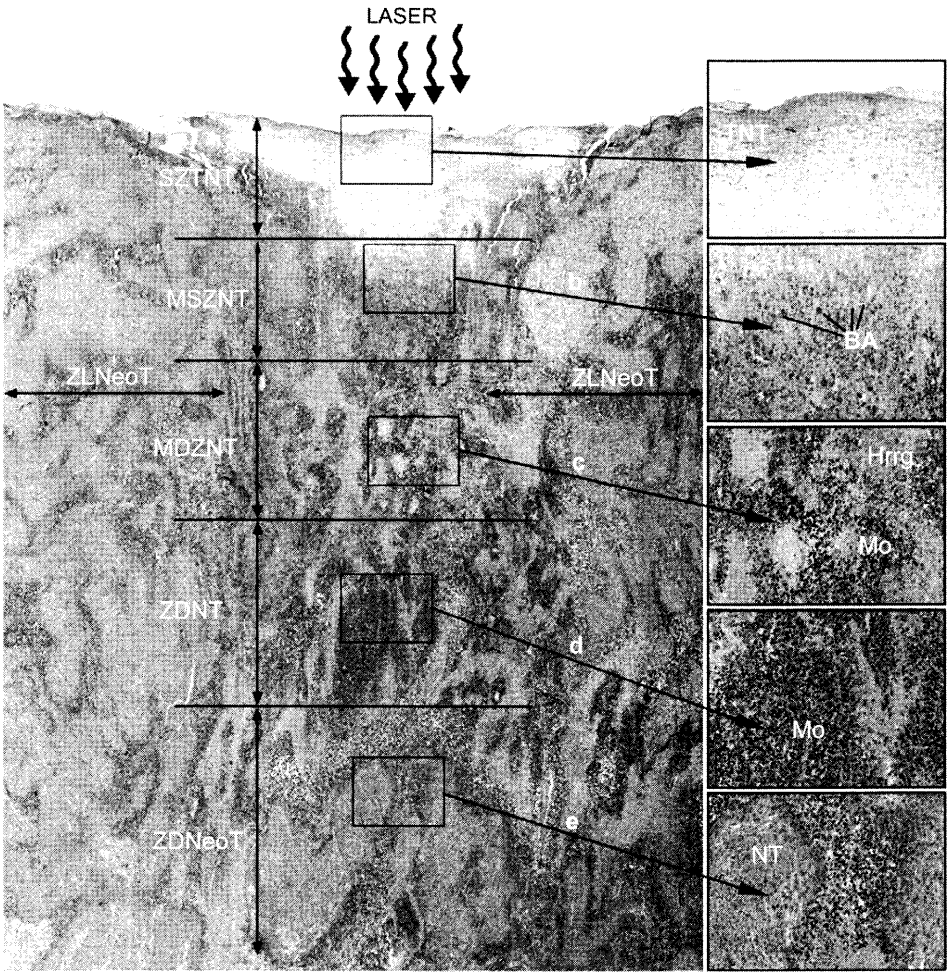

Fig. 4. Classification of the tissue lesions detected in solid myeloid *Graffi* tumor in hamsters after local treatment with gold nanoparticles (40nm) and laser irradiation: (a) SZTNT – Superficial zone of total necrotic tissue; (b) MSZNT - Middle superficial zone of necrotic tissue; (c) MDZNT – Middle deep zone of necrotic tissue; (d) ZDNT – Zone of deep necrotic tissue; (e) ZDNeoT and ZLNeoT – Zone of deep neoplastic tissue and Zone of lateral neoplastic tissue. Hematoxylin-eosin

The conducted studies showed that the combination of treatment with gold nanoparticles and laser effectively suppressed the tumor tissue growth and had temporary positive effects on the reduction of tumor cell mass within the solid tumors. Pathohistological studies clearly highlighted separate zones of nanothermolysis in the tumor tissue, which could help to improve the parameters of the nanoparticles and laser system in future experiments, aiming the optimal conditions for total destruction of the tumor cells in lateral and deep zones. These neoplastic cells remained viable which allowed the lateral tumor growth and explained the temporary inhibition on the tumor growth.

The results obtained showed that application of plasmonically activated gold nanoparticles for *in vivo* treatment of *Graffi* tumor in hamsters demonstrate considerable antitumor effect and have the potential to be used for local treatment of small solid tumors.

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