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Photo-thermal tumor ablation in mice using near infrared-absorbing nanoparticles

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Abstract

The following study examines the feasibility of nanoshell-assisted photo-thermal therapy (NAPT). This technique takes advantage of the strong near infrared (NIR) absorption of nanoshells, a new class of gold nanoparticles with tunable optical absorptivities that can undergo passive extravasation from the abnormal tumor vasculature due to their nanoscale size. Tumors were grown in immune-competent mice by subcutaneous injection of murine colon carcinoma cells (CT26.WT). Polyethylene glycol (PEG) coated nanoshells ($\approx$130 nm diameter) with peak optical absorption in the NIR were intravenously injected and allowed to circulate for 6 h. Tumors were then illuminated with a diode laser (808 nm, 4 W/cm^2, 3 min). All such treated tumors abated and treated mice appeared healthy and tumor free $>$90 days later. Control animals and additional sham-treatment animals (laser treatment without nanoshell injection) were euthanized when tumors grew to a predetermined size, which occurred 6–19 days post-treatment. This simple, non-invasive procedure shows great promise as a technique for selective photo-thermal tumor ablation.

Keywords: Hyperthermia; Nanotechnology; Nanoshell; Near infrared; Laser; Minimally invasive therapy

1. Introduction

The destruction of solid tumors using hyperthermia has been under investigation for some time. Previously investigated thermal therapies have employed a variety of heat sources including laser light, [1–4] focused ultrasound, [5] and microwaves [6–8]. The benefits of thermal therapeutics over conventional resection are numerous; most approaches are minimally or non-invasive, relatively simple to perform, and have the potential of treating embedded tumors in vital regions where surgical resection is not feasible. However, in order to reach underlying tumors or to treat large tumors, the activating energy source must sufficiently penetrate healthy tissues. Unfortunately, simple heating techniques have trouble discriminating between tumors and surrounding healthy tissues, and often heat intervening tissue

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between the source and the target site. Several groups have investigated treatment of tumors via hyperthermia using deep penetrating near infrared (NIR) lasers with or without contrast enhancing agents (indocyanine green); however, success with current systems has been modest [1,9,10].

This study introduces a new laser-induced thermal therapy employing systemically delivered, NIR absorbing nanoparticles called nanoshells. Nanoshells are a new class of optically tunable nanoparticles composed of a dielectric core (silica) coated with an ultrathin metallic layer (gold) [11]. By adjusting the relative core and shell thickness, nanoshells can be manufactured to absorb or scatter light at a desired wavelength across visible and NIR wavelengths. This optical tunability permits the fabrication of nanoshells with a peak optical absorption in the NIR, a region of light where optical penetration through tissue is optimal [12]. Furthermore, the metal shell of the nanoshell converts absorbed light to heat with an efficacy and stability that far exceeds that of conventional dyes investigated earlier. Nanoshells possess absorption cross sections that are six orders of magnitude larger than indocyanine green, making this material a much stronger NIR absorber, and therefore a more effective photothermal coupling agent [13]. In addition, a nanoshell's absorption properties are dependent upon the material's rigid metallic structure rather than the more labile molecular orbital electronic transitions of conventional dyes. This makes nanoshells less susceptible to photobleaching, a problem commonly associated with dyes. The efficacy of nanoshells as a NIR absorber has already been demonstrated in a series of in vivo magnetic resonance thermal imaging (MRTI) studies examining temperature profiles of nanoshell-loaded tumors irradiated with NIR light. These studies found nanoshells absorb NIR light and generate increased temperatures sufficient to produce irreversible photo-thermal damage to subcutaneous tumors [14].

As a biomaterial, nanoshells are composed of elements generally understood to be biocompatible. The metal surface of the nanoshells employed here consists of gold, an inert metal well known for its biocompatibility. To further improve biocompatibility, 'stealthing' polymers like poly(ethylene glycol) (PEG) can be grafted to nanoshell surfaces using simple molecular self assembly techniques [15]. It has been demonstrated that stealthing liposomes as well as other biomolecules and materials with PEG suppresses immunogenic responses, improving blood circulation times and overall material/implant performance [16,17].

Substantial prior research has investigated the delivery of macromolecules and small particles through the tumor vasculature. It has been demonstrated that macromolecules and small particles in the 60–400 nm size range will extravasate and accumulate in tumors [18–22] via a passive mechanism referred to as the 'enhanced permeability and retention' (EPR) effect [23,24]. This behavior has been attributed to the leaky nature of tumor vessels, which contain wide interendothelial junctions, an incomplete or absent basement membrane, a dysfunctional lymphatic system, and large numbers of transendothelial channels [19,25]. NIR absorbing nanoshells can be manufactured within size ranges that should demonstrate the same preferential, size-dependent accumulation in tumors via the EPR effect.

This report describes a new technique that exploits the optical, chemical and physical properties of nanoshells in conjunction with the deep penetrating properties of NIR light for a targeted, minimally invasive photothermal therapy. The principle goal of this project was to determine the efficacy of NAPT using nanoshells fabricated of an appropriate size for extravasation into tumors with optical absorption in the NIR. Subcutaneous tumors were grown in mice, solutions of PEG-coated (or 'PEGylated') nanoshells were injected intravenously, and accumulation within the tumor was monitored. Nanoshell-treated tumors and nanoshell-free shams were then exposed to NIR light. Resultant heating and therapeutic efficacy was assessed via surface temperature measurements, monitoring of tumor growth/regression, and animal survival times.

2. Materials and methods

2.1. Synthesis of thiolated polyethylene glycol (PEG-SH)

PEG with a terminal thiol group (PEG-SH) was synthesized by reacting PEG-amine (MW 5000, chromatographically pure, Nektar) with

2-iminothiolane (Sigma) for 1 hour. The product was then dialyzed (MWCO 3500 dialysis cassette, Pierce) against deionized (DI) H_2O for 6–8 hours to remove excess reagent. The PEG-SH yield was determined colorimetrically at 412 nm after reaction with Ellman's Reagent (5,5′-Dithio-bis(2-Nitrobenzoic Acid), Sigma). The product was stored in aliquots at −20 °C.

2.2. Gold-silica nanoshell fabrication

Nanoshells were fabricated as previously described [26]. Briefly, 110 nm diameter silica nanoparticles were obtained (MP-1040, Nissan Chemical America Corporation) and suspended in ethanol. The particle surface was then terminated with amine groups by reaction with 3-aminopropyltriethoxysilane (APTES, 97 + %, Avocado Research Chemicals, Ltd). Very small gold colloid (1–3 nm dia.) was grown using the method of Duff, et al. [27]. This colloid was aged for 4–14 days at 6 °C and then concentrated using a rotary evaporator. The aminated silica particles were then added to the gold colloid suspension. Gold colloid adsorbs to the amine groups on the silica surface resulting in a silica nanoparticle covered with islands of gold colloid. Gold-silica nanoshells were then grown by reacting $HAuCl_4$ (Sigma-Aldrich) with the silica-colloid particles in the presence of formaldehyde. This process reduces additional gold onto the colloid adsorbed the silica particle surface. These colloidal islands serve as nucleation sites, causing the surface colloid to grow, eventually coalescing with neighboring colloid, to form a complete metal shell. Nanoshell optical properties were assessed using a UV–Vis spectrophotometer (Genesys 5, Spectronic, Inc.). The resulting nanoshell solutions possessed an 8–10 nm thick gold shell, generating a peak optical absorption at 805–810 nm. Nanoshell surfaces were coated with PEG by combining PEG-SH (25 μM final concentration) with nanoshells (8×10^9 nanoshells/ml) in DI water for 1 hr, followed by centrifugation to remove residual PEG-SH from the nanoshell formulation. Prior to injection, PEGylated nanoshells were resuspended at 2.0×10^{11} nanoshells/ml concentration in sterile 0.9% saline solution and sterilized using a 0.22 μm syringe filter.

2.3. Tumor inoculation

All animals were handled and cared for in accordance with the 'Guide for the Care and Use of Laboratory Animals' [28] 25 female albino BALB/cAnNHsd mice (5–6 weeks age, 15–20 g, Harlan Sprague–Dawley, Indianapolis, IN) were obtained. Each was shaved on the right dorsal flank prior to subcutaneous inoculation with 1.5×10^5 (50 μl injection volume) CT26.WT murine colon carcinoma tumor cells (ATCC) [29].

2.4. Nanoshell injection and laser treatment

Mice were selected for treatment when the subcutaneous tumors reached 3–5.5 mm diameter as measured with a digital caliper (8–16 days post-inoculation). A 5.5 mm tumor diameter was selected as the maximum possible treatment size due to the spot size of the collimated laser. Seven of the mice had two tumors, and 17 had one. These were randomly distributed between 3 groups. Prior to each nanoshell injection, each mouse was anesthetized via intraperitoneal avertin injection (120 mg/kg, or 20 μl/g body weight, 1.2% solution). 100 μl of the 2.4×10^{11} nanoshells/ml solution was then injected via the tail vein for mice in the treatment group. For the sham treatment group, a 100 μl 0.9% sterile saline injection was substituted for the nanoshells suspension. The control group received no intravenous injections or subsequent laser treatment. Laser treatments on the nanoshell treatment and sham treatment groups were performed 6 h after injection to allow the systemically delivered nanoshells time to accumulate in the tumors. The skin at the tumor site was swabbed with polyethylene glycol (Aldrich) as an index matching agent to maximize penetration of light into the tissue. Tumors in the nanoshell and sham treatment groups were exposed to NIR light (808 nm diode laser, 800 mW, Power Technologies, Alexander, AR; 600 μm fiber optic patch cable to a collimating lens, 1:1 Optical Imaging Accessory, Coherent, Inc., Santa Clara, CA) at 4 W/cm^2 for 3 min. An additional laser treatment was performed on one mouse in the nanoshell treatment group at a spot 3 mm away from the tumor to determine if abnormal heating occurred in nearby normal tissue where nanoshells should not have accumulated.

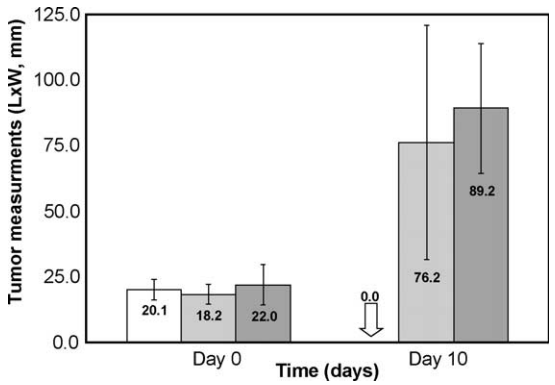

Fig. 1. Mean tumor size measured on treatment day and 10 days later for 25 tumors. All tumors which were treated using NAPT showed complete necrosis by day 10. One standard deviation is shown. NAPT treatment group ($n = 7$), sham treatment group ($n = 8$), untreated controls ($n = 9$).

During the NIR treatments, the cutaneous temperature was measured with a handheld infrared thermometer (Omegascope OS530L-CF, Omega Engineering, Stamford, CT) which integrates thermal measurements across a 5 mm diameter spot. Post-treatment tumor size measurements were taken daily using a digital caliper. Survival time was also monitored, and animals were euthanized via trifluoroethane asphyxiation when tumor diameter reached 10 mm.

Statistical analysis was performed on the three groups by comparing the means of the tumor sizes at day 0 and day 10 post-treatment and computing the level of significant difference as measured by the p value. Similarly, p values were computed to compare the mean surface temperatures measured for the treatment and sham treatment groups. The survival time comparison for the three groups was augmented with a Kaplan-Meier survival analysis which generates confidence intervals for survival times.

3. Results and discussion

Surface temperature measurements were obtained during each NIR laser treatment for the 15 mice in the nanoshell and sham treatment groups as described. The surface temperature is a surrogate for temperatures achieved within the tumors of the NAPT treatment group as well as a measurement of thermal absorption by skin and tissue in both laser treated groups. By 30 s, the mean temperature of

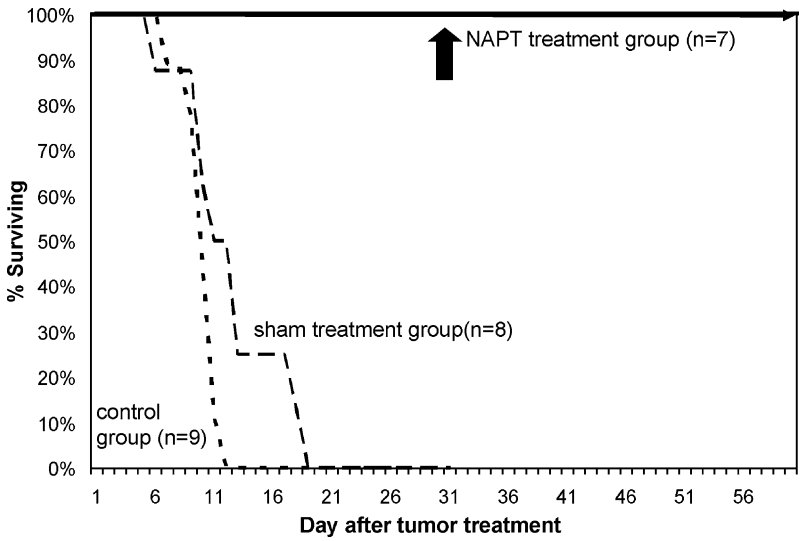

Fig. 2. A survival time plot for the three groups for the first 60 days. The mean survival time for the control group was 10.1 days with a 95% confidence interval of 9.2–11.1 days. The mean survival time for the sham group was 12.5 days with a 95% confidence interval of 9.5–15.5 days. By 18 days the mean survival time of the treatment group was significantly higher ($P < 0.001$) compared to either the control or the sham group.

the laser/nanoshell treated tumors (~50 °C) was significantly higher than NIR treated but nanoshell-free controls ($P < 0.0001$). For comparison, a NIR treatment was applied to a healthy skin area several mm away from the tumor site on a nanoshell treated animal. In this non-tumor location, thermal responses were identical to those observed in animals that had not received a nanoshell treatment. This suggested that a preferential accumulation of nanoshells via the EPR effect was sufficient to generate a therapeutic result at the tumor site but not sufficient to affect damage to surrounding healthy tissues.

Tumor size and animal survival were monitored for 90 days following treatment. Within 10 days of nanoshell treatment, complete resorption of the tumor was observed. At 90 + days post-treatment, all mice remained healthy and free of tumors. Meanwhile, tumors in both the sham and control groups continued to grow rapidly (Fig. 1). Mice were euthanized when tumor diameter exceeded 10 mm (corresponding to >5% body weight). All control mice were euthanized by day 12 (mean survival time 10.1 days) and all mice in the sham group were euthanized by day 19 (mean survival time 12.5 days) (Fig. 2).

The goals of this study were to determine if nanoshell-assisted photo-thermal ablation would arrest the growth of tumors grown in mice. NAPT-treated tumors displayed complete regression and these mice remained healthy and tumor-free >90 days following treatment. Further research is ongoing to optimize treatment parameters and to investigate the mechanisms involved in tumor regression following NAPT treatment. These studies will include analysis of nanoshell biodistribution and histological assessment of tissue damage.

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