Feasibility Study of Particle-Assisted Laser Ablation of Brain Tumors in Orthotopic Canine Model

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Abstract

We report on a pilot study showing a proof of concept for the passive delivery of nanoshells to an orthotopic tumor where they induce a local, confined therapeutic response distinct from that of normal brain resulting in the photothermal ablation of canine transmissible venereal tumor (cTVT) in a canine brain model. cTVT fragments grown in severe combined immunodeficient mice were successfully inoculated in the parietal lobe of immunosuppressed, mixed-breed hound dogs. A single dose of near-IR (NIR)-absorbing, 150-nm nanoshells was infused i.v. and allowed time to passively accumulate in the intracranial tumors, which served as a proxy for an orthotopic brain metastasis. The nanoshells accumulated within the intracranial cTVT, suggesting that its neovasculature represented an interruption of the normal blood-brain barrier. Tumors were thermally ablated by percutaneous, optical fiber–delivered, NIR radiation using a 3.5-W average, 3-minute laser dose at 808 nm that selectively elevated the temperature of tumor tissue to 65.8 ± 1.2°C. Identical laser doses applied to normal white and gray matter on the contralateral side of the brain yielded sublethal temperatures of 48.6 ± 1.1°C. The laser dose was designed to minimize thermal damage to normal brain tissue in the absence of nanoshells and compensate for variability in the accumulation of nanoshells in tumor. Postmortem histopathology of treated brain sections showed the effectiveness and selectivity of the nanoshell-assisted thermal ablation. [Cancer Res 2009;69(4):1659–67]

Introduction

The incidence of brain tumors in the United States, including primary and metastases of other cancers, is estimated to be around 200,000 per year (1). The incidence of brain tumors is reported to be increasing, although this may be due to enhanced detection techniques (2). Primary brain neoplasms account for ~10% of the total incidence, the majority of which (~75%) are classified as glioma or meningioma. The majority of brain tumors by far are metastases that most commonly arise from lung cancer (40–50%), breast cancer (15–25%), and melanoma (5–20%; ref. 3).

The management of brain tumors can be divided into symptomatic and/or therapeutic strategies. Symptomatic therapies primarily aim to decrease peritumoral edema and prevent recurrent seizures. Therapeutic modalities, on the other hand, aim to eradicate the brain cancer. The currently accepted treatment modalities for tumors of the brain include surgery, whole-brain radiotherapy, stereotactic radiosurgery (SRS), chemotherapy, or any combination of the above (3). Treatment regimens must take into account patient age, functional status, extent of systemic disease, tumor type and size, the number of tumors present, and prior therapies. For solitary masses under 3 cm in diameter, SRS has become the preferred treatment method. SRS uses multiple convergent beams to treat a discrete target area. Although it has eliminated surgical comorbidities by eliminating the need for an invasive intracranial procedure, radiotherapy still has significant complications, including early treatment-induced edema, seizures, and delayed radiation necrosis. There exists, then, the need for an effective treatment of solitary tumors that can avoid some or all of these problems.

We are investigating a particle-based, thermal ablative technique that offers significant promise in this area due to its potential to provide a high level of precision for tumor elimination while limiting or avoiding altogether radiotherapy-related complications. Particles are delivered i.v. and allowed to accumulate in the tumor by virtue of the enhanced permeability and retention effect, which characterizes the propensity for tumors to accumulate small particles and macromolecules from blood resulting from their poorly organized and fenestrated vasculature (4–6). Nanoshells (AuroShell particles, Nanospectra Biosciences, Inc.) used in this study are composed of a dielectric silica core encased in a thin gold shell with a total diameter of 144 to 150 nm. Nanoshells possess a plasmon resonance that imparts a large extinction coefficient over a relatively narrow band of the electromagnetic spectrum. The location of the plasmon resonance is dependent on the relative diameters of dielectric core and the gold shell (7, 8). By tuning the nanoshell resonance to the near-IR (NIR) region of the spectrum, the optical extinction of any tissue in which they accumulate may be greatly enhanced. Thus, illumination via a similarly tuned laser source may be used to preferentially heat nanoshell-bearing tissues.

Systemically administered nanoshells have previously shown a high selectivity for tumors and no extravasation into normal tissue, with particles cleared from the bloodstream by the reticuloendothelial system (9). When tumors accumulate nanoshells via enhanced permeability and retention, they may then be efficiently ablated with high selectivity over surrounding healthy tissues as seen in the effective elimination of solid tumors in several animal models (10–12). In addition, nanoshells have recently been used to induce mild hyperthermia in tumors and consequently enhance their susceptibility to radiotherapy in a human colorectal tumor model (13).
Here, we present the results of a pilot study for the nanoshell-
enhanced ablation in a canine transmissible venereal tumor (cTVT)
brain tumor model. The two objectives of this study were to show
the feasibility of ablation using a single dose of i.v. infused particles
followed by a fixed laser dosimetry and to evaluate the need for
real-time thermal monitoring to adjust laser dosimetry during the
procedure.

Materials and Methods

Nanoshell fabrication. Nanoshell fabrication was based on the method
of Oldenburg and colleagues (14). Briefly, gold colloids 1 to 3 nm in diameter
were grown over an agitated, 120 ± 12 nm core of colloidal silica
(Precision Colloids, LLC) using the method of Duff and Baiker (15). Gold
colloid adsorbed to the amine groups on the surface of the silica core to
form nucleating sites, which were then further reacted with HAuCl₄ in the
presence of formaldehyde. This caused the surface colloid to grow and
coalesce, ultimately forming a complete metal shell. Finished particles
possessed a 12- to 15-nm-thick shell that resulted in an optical absorption
peak between 780 and 800 nm. Thiolated polyethylene glycol (SH-PEG;
Laysan Bio) was then assembled onto nanoshell surfaces by combining 1
mmol/L SH-PEG and nanoshells in a ratio of 1.5 μL/mL in deionized H₂O
for 12 h followed by diafiltration to remove the excess SH-PEG. Particles
were then suspended in 10% trehalose solution to create an iso-osmotic
solution for injection and concentrated by transverse flow filtration to an
extinction of 100 ± 5 absorbance (at 800 nm) to reduce the infused fluid
volume. Nanoshells were fabricated under clean conditions in a class 100
clean room and passed through a 0.45-μm filter.

We based the nanoshell dosing level on extensive prior research in
rodents.⁵ We arrived at an optimal nanoshell dose determined from NAA-
derived nanoshell accumulation in allografted tumors. The optimal
nanoshell dose, corresponding to 1.25×9 nanoshells/gram of body weight,
was scaled up for this study by the estimated blood volume for mongrel
dogs versus mice (5.2 versus 4.5 mL/kg, ref. 16).

cTVT propagation. All animals were handled in accordance with
Association for Assessment and Accreditation of Laboratory Animal Care
and Office of Laboratory Animal Welfare guidelines under the direction of
the Institutional Animal Care and Use Committees at Nanospectra
Biosciences and The University of Texas M. D. Anderson Cancer Center.
cTVT is a naturally occurring transplantable round cell tumor in dogs (17, 18). Because cTVT spontaneously regresses in healthy dogs, its growth and
propagation requires an immunosuppressed host. Fresh cTVT fragments
were used for inoculation until sacrifice. Tumors were allowed to grow until their major
axis approached 1 cm in length, typically at 6 to 8 wk when their presence
was confirmed using magnetic resonance–dynamic contrast enhancement
(MR-DCE). When an appropriately sized tumor was confirmed, the dog was
scheduled for nanoshell infusion and laser treatment.

Laser and water-cooled, percutaneous laser applicator. NIR
radiation was provided by a high-power diode laser (15 plus, Diomed, Inc.)
delivered percutaneously via a water-cooled diffusing optical fiber
applicator, the Visualase Cooled Laser Applicator System (VCLAS),
shown in Fig. 1B. Calibration of the laser output power was accomplished using a
calibrated integrating sphere optometer (P9710-2, Gigahertz-Optik, GmbH).
The laser was a 15-W gallium arsenide diode unit emitting at 810 ± 20 nm in
continuous or pulsed modes. This wavelength is within the NIR
“therapeutic window” (700–1,300 nm) that minimizes endogenous
absorption by oxyhemoglobin and deoxyhemoglobin, maximizing optical
penetration through vascularized tissue (19, 20), and is simultaneously near
absorption peak of the nanoshells. Within the dual lumen of the VCLAS
was an optical fiber terminated with a 1-cm-long isotropic diffusing tip. The
diffuser provides highly uniform emission longitudinally and radially. The
VCLAS permitted minimally invasive access to orthotopic tumors and
the water-cooled applicator is effective at increasing the treatment depth by
cooling tissue proximate to the fiber, which would normally be the first to
denature, limiting subsequent light penetration.

MRI temperature monitoring and Visualase thermal mapping
system. MRI was used to monitor spatially resolved temperatures in vivo
based on the temperature-dependent proton resonance frequency shift.
Complex phase-difference images from a temperature-sensitive interleaved
gradient-echo, echo-planar imaging sequence were processed to display
temperature by the Visualase system (21). The system interfaces with the
MRI via Ethernet and uses information derived from temperature-sensitive
MRI images to provide near real-time temperature mapping of the MR
images (22). The system allows an operator to closely follow the thermal
therapy by monitoring real-time MR images simultaneously overlaid with a
false-color map of temperature and modeled Arrhenius thermal ablation
zones (17).

Photothermal ablation. The laser dosimetry used for all of the cases
presented here was developed in previous studies in mice and rats. We
found that 3.5 W of 808-nm light, delivered from the 1-cm isotropic diffusing
fiber with a duty factor of 75% for 3 to 4 min, was at or just below the
threshold for producing thermal ablation in vascularized tissue. We

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⁵ D.P. O’Neal and K. Gill-Sharp, unpublished data.
attribute the difference in thermal ablation volumes between the nanoshell and vehicle control brains detailed here to the presence or absence of nanoshell accumulation.

The s.c. cTVT tumors were monitored in the dogs as an index for brain tumor growth. When these tumors became palpable, the dogs were anesthetized and taken for MR evaluation of their brain inoculations. Brain tumors were detected by MR-DCE using gadopentetate dimeglumine (Magnevist, Bayer Pharmaceuticals) as a contrast agent. The contrast agent changes the homogeneity of the magnetic field in the surrounding tissue and results in a marked decrease in $T2^*$ relaxation time. As the bolus passes through a region of interest, the signal from that region is attenuated as a result of spin dephasing. The signal returns to normal after the bolus has passed (23).

Dogs receiving nanoshells were i.v. infused with 5.2 mL/kg of 100 absorbance nanoshells at a rate of 2 mL/min for 5 min, subsequently increased to 5 mL/min for the duration. Dogs used as controls received the same dose and rate of the vehicle (10% trehalose). Twenty-four hours after nanoshell infusion, dogs were anesthetized as before, the cranial incision was reopened, the Tuohy-Borst adapters were rethreaded into the Bone Anchors, and the dogs were taken to the MR suite. There, they were imaged by MR-DCE to confirm the presence and location of the previously observed tumor. Using sterile technique, the VCLAS cooled catheter was inserted through and secured by the Bone Anchor, positioning the optical fiber within the brain at the level of the tumor. A series of temperature-sensitive echo-planar imaging sequence MR images were collected at 6-s intervals, whereas the laser was activated at 3-W output for 30 s. Localized heating was measured by this imaging sequence using the real-time thermal mapping system, which permitted verification of the location of the optical fiber diffusing tip relative to the desired target. The catheter was adjusted as necessary to bring it in good proximity to the tumor. We carried out the photothermal treatment in the same manner, recording magnetic resonance thermal imaging (MRTI) data, which were displayed in real time as the laser was energized at 3.5 W for 3 min. After laser treatment of the tumor, the laser catheter was removed and reinserted through the contralateral Bone Anchor into normal brain and an identical laser treatment was performed. After photothermal treatment, another series of MR-DCE images were recorded to assess acute changes in blood perfusion in the treated areas of the brain. Forty-eight hours after laser treatment, dogs were anesthetized and imaged to assess short-term resolution of the thermal injury. The dogs were then sacrificed under anesthesia for necropsy examination and tissue harvest. Harvested tumor and brain tissue samples were obtained for histopathology and neutron activation analysis for gold content as a measure of particle uptake. The intact brain and tumor samples were fixed by immersion in a 10% buffered formalin solution for a minimum of 96 h before slicing and documentation of the extent of the thermal damage.

**Neutron activation analysis.** Tissue samples containing gold-bearing nanoshells were assayed *ex vivo* using neutron activation (or elemental) analysis (EA). EA for these studies, conducted by the Nuclear Engineering Teaching Laboratory at the University of Texas at Austin, uses neutron bombardment to excite stable gold $\gamma$Au$^{197}$ to the radioactive $\gamma$Au$^{198}$. This radioactive isotope decays with a half-life of 2.7 d, emitting characteristic 412 keV $\gamma$-rays that were used to quantify gold content. We used the EA-measured gold and tissue mass to derive gold concentrations in tested tissues. Calculating the expected mass of gold per nanoshell yields an estimate of the number of particles present.

**Results**

The key features of the nanoshell infusion and laser treatment are summarized in Table 1. Of the nine dogs inoculated with cTVT, only five grew intracranial tumors. Of those with tumors, four received nanoshells and photothermal ablation (dogs 1–4 in Table 1) and one received laser illumination but no nanoshells as a control (dog 5 in Table 1). Of the four dogs that did not grow tumors, three were withdrawn from the study and one served as a healthy control (dog 6 in Table 1) for photothermal ablation in the
brain. There were no significant neurologic issues after any intracranial procedure with the exception of one dog, which had a single seizure during recovery from anesthesia immediately following laser treatment. This dog recovered immediately and stayed free of seizures or other neurologic problems for the remainder of the study. No signs of neurologic damage were observed in other dogs after laser treatment and before sacrifice.

Laser illumination was applied from a single fiber insertion, although in two cases (dogs 3 and 6 in Table 1) the fiber was retracted within the cooled catheter to treat multiple sites along the fiber track. In two cases (dogs 2 and 3 in Table 1), we observed hemorrhage during the laser illumination of the nontumored hemisphere. In both cases, the hemorrhage occurred at the base of a sulcus and seemed to result from the insertion of the optical fiber catheter rather than as a result of laser illumination. The maximum temperature achieved in any MRTI pixel is indicated in Table 1.

The extent of the thermal coagulonecrosis was evaluated using H&E-stained sections of formalin-fixed brain tissue approximately 48 hours after the thermal insult. Unlike other tissues in the body, tissues of the central nervous system characteristically undergo rapidly liquefactive necrosis following a lethal insult (24). Immediately following lethal thermal irradiation, the brain and other tissues typically undergo coagulative necrosis characterized by preservation of the previous tissue structure and architecture. The 48-hour time period was found in previous studies to allow maximum progression of the necrotic process before liquefaction and loss of histologic evidence of prior normal tissue structures and tumor.

We will discuss two relevant cases in detail: dog 4, which had a nanoshell-assisted tumor photothermal treatment, and dog 5, a control case that had an identical photothermal treatment without nanoshells. Figure 2 shows the results of laser treatment of dog 4. Figure 2A is an axial MR-DCE image of the same plane taken 48 hours after laser treatment, immediately before sacrifice. The single laser treatment of the inferior of the two tumor lobes is easily distinguished from the untreated superior lobe as indicated by the arrow. The superior lobe takes up the Magnevist contrast agent, indicating intact blood perfusion. The inferior lobe shows only a ring of contrast, indicating the presence of edema around the periphery of the ablated tumor lobe. The track of the laser catheter and the laser treatment of the normal brain in the left hemisphere produced insufficient damage to be seen in these images. The superior lobe of the tumor was left untreated to provide means of comparison in the MR-DCE imaging and to permit harvest of intact nanoshell-containing tumor for EA analysis.

Figure 2C and D shows gross pathology transverse slices of the left and right hemispheres, respectively, of the brain shown in Fig. 2A and B. Arrows indicate the location of the passage of the laser catheter into the plane of the image. The left hemisphere in Fig. 2C shows a small damage zone, indicating the passage of the optical fiber catheter, but no thermal damage sequelae. The right hemisphere in Fig. 2D shows that thermal damage has been confined to the tumor and an area immediately adjacent to it. Significantly, the path of the laser catheter was caudal and lateral to the ablated tumor at this plane as indicated by the arrow, showing that the isotropically irradiating laser fiber was external to the tumor. Caudal to the fiber, the normal brain tissue has remained intact. Damage rostral (right in the image) to the fiber, by contrast, indicates complete thermal ablation of the inferior lobe of the tumor. The original cTWT is shown circumscribed by the inner ring, the zone of thermal ablation and complete cell death by the middle ring, and a region of edema by the outer ring. From histopathology of all of the treated dogs, we noted cellular necrosis extended from 0.5 to 4 mm outward from the identifiable tumor boundary, indicating thermal diffusion from the particle-ablated areas.

Figure 3 shows the clinical results of an identical laser treatment on control dog (dog 5 in Table 1) that was given a blood-volume comparable infusion of the 10% trehalose vehicle without nanoshells. Figure 3A is an axial view of a MR-DCE image at the plane of the inoculated tumor taken ~1 hour before the nanoshell infusion.
The tumor has two connected lobes (indicated by arrows) along the inoculation track. Figure 3B is a MR-DCE image of the same plane taken 48 hours after laser treatment. The single laser treatment has resulted in a small ablation zone lateral to the fiber track indicated by the arrow as a small dark region. Fig. 3C and D shows gross pathology coronal slices of the left and right hemispheres, respectively, of the brain shown in Fig. 3A and B. Arrows indicate the location of the passage of the laser catheter into the plane of Figure 3. Laser treatment of cTVT in the brain of dog 5. A, arrows, MR-DCE axial image of dog brain showing contrast enhancement of bilobed tumor along inoculation track. B, arrow, MR-DCE axial image of dog brain after laser treatment showing ablation of inferior lobe. C, control cerebral hemisphere showing no ablation in normal brain. Arrow, fiber insertion point. D, ablation zones (inner ring = boundary of tumor, middle ring = boundary of thermal necrosis, outer ring = boundary of zone of edema) around nanoshell-containing tumor. Arrow, fiber insertion point.
the image, medial to the ablation zone in both cases. Zones of thermal ablation and complete cell death are circumscribed by the inner ring and surrounding regions of edema by the outer ring. The damage zones in this case are roughly comparable with thermal damage being done to both normal brain and cTVT.

Discussion

We have presented here a pilot study showing a proof of concept that nanoshells combined with laser-based thermotherapy can be effective for highly specific brain tumor ablation. The primary goal of this study was to evaluate the feasibility of the treatment. Consequently, therapeutic efficacy that would require a priori knowledge of nanoshell accumulation in the target tumor as well as laser dose optimization for treatment of the entire tumor and interactive adjustment of the laser exposure as opposed to a fixed exposure (for reproducibility) was not addressed and awaits more detailed research. We have shown that passively delivered, 150-nm nanoshells specifically accumulate in the cTVT allograft brain tumor model and that laser activation of the nanoshells results in selective photothermal ablation of tumor tissue using laser fluences that are tissue sparing in the absence of nanoshells.

The surgery, nanoshell infusion, MRI, and laser treatment portions of this study proceeded relatively smoothly. The largest difficulty proved to be the reliability of the inoculation and growth of the cTVT tumors in dog. Of the nine inoculations, five succeeded in brain, five succeeded s.c., but only three succeeded in both locations. The cTVT used in this study was harvested from and consistently passed successfully through SCID mice, so it is likely that the problem with inoculation had more to do with inadequate immunosuppression of the dogs than with tumor viability.

The nanoshell particles used in this study were designed to absorb optical radiation in the vicinity of 800 nm. The selective accumulation of these particles in the neovasculature of the growing cTVT had the effect of increasing the local optical extinction leading to increased deposition of photon energy in neovascularized tissue leading to tissue-specific ablation. The high gold concentration in viable cTVT additionally shows that cTVT microvasculature possesses pores or fenestrations into which the 150-nm nanoshells may be inserted (25). Primary glioblastomas also possess microvasculature pores, which may, depending on their size and the specific tumor type, be exploited by PEGylated nanoparticles (26). There is no evidence that 150-nm nanoshells are endocytosed by tumor cells; transmission electron microscopy analysis indicates their accumulation along capillary walls.

The graph in Fig. 4 compares EA measurements of tissue gold content for cTVT tissue and normal canine white matter. Gold concentrations in untreated, viable cTVT taken from brain was measured at 22.5 ± 24.0 μg/g of tissue weight, 2.2 times the 10.3 ± 5.6 μg/g concentration in cTVT inoculated s.c. on the flank. Also of interest is the gold concentration of 11.6 ± 10.9 μg/g remaining in ablated portions of tumor 48 hours after laser treatment. Two samples of white matter taken from dogs inoculated with nanoshells showed a gold concentration of 0.144 ± 0.143 μg/g, indicating a baseline nanoshell concentration 156-fold below that in tumor.

The 15-nm-thick gold shell over the 120-nm-diameter silica core implies a gold mass of 1.66e−14 g/nanoshell. The EA data in Fig. 4 thus suggest nanoshell concentrations of 1.36e9/g tissue weight for cTVT in brain, 6.18e8/g in cTVT in flank, 6.96e8/g in ablated cTVT, and 8.65e6/g in normal brain. This has important implications for the laser dosimetry in that nanoshells provide enhanced optical absorption at the therapeutic laser wavelength. Although the 800-nm absorption peak for nanoshells falls within the therapeutic window where endogenous optical attenuation is at a minimum, hemoglobin is still a substantial absorber with an absorption coefficient μₐ = 0.5 cm⁻¹ (assuming a 6.5% tissue blood volume). Nanoshells, which both scatter and absorb light, add to the optical extinction in tumor tissue where they accumulate. The nanoshell concentrations above add 0.51 ± 0.54 absorbance to the cTVT extinction in brain and 0.0033 ± 0.0033 absorbance to normal
white matter. Although this analysis would clearly benefit from a larger sample size, it is nevertheless clear that the enhanced optical extinction resulting from the accumulation of nanoshells within the cTVT reduces the photon mean free path by $f = 0.85$ cm, substantially confining the source of the resultant heat to tissue containing nanoshells.

In this study, we have endeavored to handle the dogs and tumors consistently and to maintain a rigorous pretreatment regimen. Nevertheless, nanoshell accumulation in intracranial cTVT varied widely, albeit 156-fold greater than in normal white matter. In spite of the variation in nanoshell accumulation indicated in Table 1, the plateau temperatures for intracranial tumors for which nanoshells had been infused [$65.8 \pm 4.1^\circ C (n = 4)]$ were greater than the tumor for which only the test vehicle had been infused ($53.1^\circ C$) and statistically distinct from the plateau temperatures for white matter in all cases. The difference in plateau temperatures between the nanoshell tumors and the contralateral white matter was $14.9 \pm 6.8^\circ C (n = 4)$. The statistics for the peak temperatures in normal white matter bears examination. As may be noted in Table 1, the plateau temperatures for white matter excluding dog 2 are a subablative $48.6 \pm 1.1^\circ C (n = 5)$; refs. 27, 28). Including dog 2, the white matter plateau temperatures rise to the ablative $50.0 \pm 3.6^\circ C (n = 6)$. The $57.1^\circ C$ white matter temperature plateau for dog 2 resulted from the hemorrhage of a blood vessel at the base of a deep sulcus through which the optical fiber had passed, thus showing the ablative potential of pooled blood in the vicinity of the

Figure 5. Comparison of thermal ablation of nanoshell-containing tumor with hyperthermia of normal white matter. A, MR-DCE sagittal image of dog 4 brain right hemisphere showing the ablated inferior tumor lobe surrounded by edema (black ring) at left and the end-of-treatment (EOT) temperature cross-section of the tumor at right. B, MR-DCE sagittal image of dog 4 left hemisphere showing native white matter at left and the EOT temperature cross-section of the white matter at right.
laser source even under irradiation conditions that were nominally subablative.

The case of dog 4 highlights two principal features of using nanoparticle-assisted laser tumor ablation: (a) that enhanced optical absorption by nanoparticles leads to tumor-specific generation of ablative levels of heat and (b) that the 3.5-W, 3-minute NIR laser dosimetry is nonablative in normal white matter and leads to a steep temperature gradient at the tumor boundary, which tends to confine thermal damage to nanoshell-containing tissue. Figure 5 shows a comparison between the results of identical laser treatments in the tumor-containing right hemisphere (Fig. 5A) and normal white matter in the left hemisphere (Fig. 5B). On the left are sagittal (rostral toward the top, caudal toward the bottom) MR T1 images of the brain and surrounding tissue. On the right are temperature measurements derived from the real-time phase images at the end of the 3-minute laser irradiation. Each line of thermal data was chosen to transsect the plane of maximum temperature for the thermal lesion in each case. The MR images are shown in reverse color for clarity. Figure 5A shows an MR image slice through the center of the ablated tumor where from Table 1 we note nanoshells had accumulated producing a gold concentration of 15.5 μg/g of tumor corresponding to ~9.34×10^7 nanoshells/gram of tumor. The graph at right illustrates that the damage margin extends to the edge of the dark ring (edema as detected by MR-DCE), 7 pixels (5.5 mm) rostral and 4 pixels (3.1 mm) caudal to the position of the optical fiber. This led to a damage zone 8.6 mm along the sagittal plane as seen in the gross pathology image in Fig. 2D, and resulted in tissue necrosis of the treated inferior lobe of the tumor surrounded by a 1- to 2-mm-wide edematous ring. Figure 5B shows a MR image slice through the plane of the optical fiber, visible as the light-colored line passing through the skull bolt at right and proceeding through the parietal lobe (leftward in the image). The graph at right indicates the subablative hyperthermia of normal white matter, which, as shown in the gross pathology image in Fig. 2C, resulted in no tissue necrosis. As indicated by Table 1, 3.5 W of NIR laser power will result in localized heating of white matter resulting in mild hyperthermia over the course of a 3-minute treatment (29). Additional tests (data not shown) at 3.8 W produced regions of heating in white matter with core temperatures ranging from 54°C to 62°C resulting in ablative lesions. It would seem, therefore, that 3.5 W of 808-nm radiation emitted from a 1-cm-long diffusing optical fiber is just below the threshold for ablative damage in unmodified brain tissue.

The enhanced vascularity of the cTVT tumor that enabled its visualization with MR-DCE also suggests an increased optical absorption due to enhanced blood volume. Simultaneously, the enhanced blood volume suggests the possibility of enhanced perfusion cooling of the tumor tissue. The thermal ablation produced in the control brain shown in Fig. 3 suggests that the increased absorption is the dominant effect—that any increase in perfusion cooling was insufficient to offset the absorption by blood of NIR photon energy from the laser.

Additional studies will be necessary to elucidate the efficacy of nanoshell-assisted photothermal ablation in different classes of tumors to add statistical power to the results presented here. In this pilot study, we have developed a procedure by which systematically infused nanoshells, in conjunction with a minimally invasive percutaneous optical fiber delivering low-dose NIR radiation, enabled us to selectively ablate orthotopically situated tumors in the brain with a minimal margin of damage to adjacent normal brain tissue. Systemic delivery of nanoshells permitted nanoshell accumulation in cTVT without restriction on tumor location, and the lack of a biochemical or metabolic interaction with tissue should make it possible for nanoshell-assisted treatment to be used in conjunction with other therapies.

Disclosure of Potential Conflicts of Interest

Nanospectra Biosciences manufactures AuroLase® Particles and has an Investigational Device Exemption from the Food and Drug Administration to develop AuroLase® Therapy for recurrent tumors of the head and neck.

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