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Gold nanorod mediated plasmonic photothermal therapy: A tool to enhance macromolecular delivery

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Abstract

Plasmonic photothermal therapy (PPTT) with gold nanostructures has been used to generate significant heat within tumors to ablate vasculature. Here we report the use of gold nanorod (GNR) mediated PPTT to induce moderate hyperthermia as a tool to enhance the delivery of macromolecules. GNRs were injected intravenously in a mouse sarcoma (S-180) tumor model. After 24 hours Evans blue dye (EBD) was injected and the right tumor was radiated with a laser diode for 10 minutes. EBD content in the right and left tumors were extracted in formamide, measured spectrophotometrically and expressed as a thermal enhancement ratio (TER). Enhanced delivery of EBD was observed (up to 1.8-fold) when tumor temperatures reached 43°C or 46°C. No statistical difference was observed between tumors at these two temperatures, though significant hemorrhage was observed in tumors and surrounding areas receiving the higher thermal dose (46°C). These results indicate that tumor directed PPTT may be used to induce moderate hyperthermia and therefore selectively increase the delivery of macromolecules with therapeutic anticancer drugs.

Keywords

Gold nanorods; Hyperthermia; Enhanced permeability and retention (EPR); Plasmonic photothermal therapy (PPTT); Permeability

It is well known that the permeability of tumor blood vessels is higher than that of tissues with a healthy morphology (Maeda et al., 2000). Enhanced permeability and retention (EPR) mainly due to large fenestrae between endothelial cells in tumor blood vessels allow for the diffusion of macromolecules out of the bloodstream enabling nanocarriers to deliver therapeutic anticancer drugs to cancerous cells (Hashizume et al., 2000; Maeda et al., 2006). Under conditions of elevated temperatures and increased blood perfusion it has been found that this tumor microvascular permeability is significantly increased (Fujiwara and

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Watanabe, 2008; Lefor et al., 1985). For example, the extravasation of Evans blue dye (EBD), a dye with high albumin affinity and therefore serving as a macromolecule indicator, as well as liposomes has been shown to be enhanced under conditions of hyperthermia (Chen et al., 2008; Gnant et al., 1999; Kong et al., 2000, 2001; Lefor et al., 1985; Matteucci et al., 2000). This is believed to be due to endothelial cell injury and thus can be used to enhance the passive delivery of nanocarriers (Chen et al., 2005; Fajardo et al., 1985; Hildebrandt et al., 2002; Xu et al., 2006).

Despite the apparent advantages of using heat for either tumor ablation or enhancing the delivery of macromolecules, clinical use of tumor hyperthermia is difficult due to limited ability to deliver sufficient heat in target regions without harming native tissue (Wust et al., 2002). More recently several laboratories have taken advantage of unique nanoscale events that occur when light is absorbed by plasmonic gold nanostructures. In brief, when light with a wavelength that matches the tunable surface plasmon resonance (SPR) of gold nanostructures meets these particles, coherent oscillations of electrons in the conduction band allow the light to be absorbed and photothermal conversion to occur (Link and El-Sayed, 2000). When such particles are localized within tissue with the intention of using this technique as a tool to induce hyperthermia, termed plasmonic photothermal therapy (PPTT), effective heating is possible (Huang et al., 2008). With PPTT many groups have achieved tumor selective temperatures from 50°C to over 70°C, well above the threshold required for vascular damage (Dickerson et al., 2008; Hirsch et al., 2003; O'Neal et al., 2004; Stern et al., 2008; von Maltzahn et al., 2009).

In the following short note, PPTT is used as a tool to induce both severe (46°C) and moderate hyperthermia (43°C). Gold nanorods (GNRs) were used in this study as they are known to have higher absorption and scattering coefficients per unit size when compared to other architectures such as spheres or shells (Jain et al., 2006). By quantification of EBD extravasation in tumors receiving PPTT, it is shown that such a technique may be used as a means of enhancing the permeability of tumor vessels and therefore enhancing the delivery of nanocarriers.

GNRs were synthesized with an SPR peak between 800-810 nm using the seed-mediated growth method (Nikoobakht and El-Sayed, 2003). After centrifugation and washing three times with deionized water, poly(ethylene glycol) (PEG) (methoxy-PEG-thiol, 5 kD, Creative PEGWorks #PLS-604) was added to the GNR suspension (optical density (OD) = 10) at a final PEG concentration of 100 μM and stirred for 1 hour. The PEG-GNR mixture was then dialyzed (3.5 K MWCO, Spectrum Labs #132594), centrifuged, washed and concentrated to remove unreacted PEG.

Mouse sarcoma S-180 cells were propagated by intraperitoneal injection (5×10^6 S-180 cells in 1 ml phosphate buffered saline (PBS)) in female CD-1 mice (4-6 weeks old) and allowed to grow until 15% weight gain was observed. Animals were then euthanized by CO_2 gas inhalation and the cells were harvested from the abdominal cavity. The cells were then washed to remove blood, diluted and subcutaneously injected into each flank of the animal (2×10^6 cells/flank in 200 μl PBS) while anesthetized with isofluorane. Tumors were then allowed to grow until average tumor volume reached 50-100 mm^3 (usually 7-10 days).

The animals were separated randomly into groups. Half received 200 μl of GNRs (9.6 mg/kg, OD = 120) and the other half saline by intravenous injection through the tail vein. After 24 hours, enough time for the GNRs to accumulate in the tumor at 1.22% injected dosed based on previous experiments and other reports in the literature (Dickerson et al., 2008), the animals were anesthetized and the areas around the tumors were shaved and swabbed with 50% propylene glycol to enhance laser penetration depth (Wang, 2002). After 20 minutes

EBD (10 mg/kg in 200 μl saline) was injected intravenously and a 33 gauge needle thermocouple (Omega #HYP0-33-1-T-G-60-SMPW-M) was inserted into the center of the tumor to monitor tumor temperatures. After roughly 10 seconds that temperature data was collected, an 808 nm fiber coupled laser diode (Oclaro #BMU6-808-02-R01) with collimating lens (Thorlabs #F810SMA-780, spot size = 7 mm) was directed over the right tumor and radiated. Two different laser powers were used in this study (1.6 and 1.2 W/cm^2) such that one group received severe and the other moderate tumor hyperthermia. After 10 minutes of radiation, the laser was turned off and tumors were allowed to cool for two minutes before removal of the temperature probe. The left tumor did not receive laser treatment to serve as an internal control.

After the animals were allowed to rest for 5 hours, enough time for the EBD to be cleared from the blood (Matsumura and Maeda, 1986), the animals were sacrificed by CO_2 inhalation. Both tumors were collected, weighed and the EBD was extracted in 1.5 ml of formamide for 48 hrs at 60°C. The EBD content was then measured spectrophotometrically at 620 nm and divided by the weight of the tumor (Matsumura and Maeda, 1986). The extravasation of EBD was then calculated as a ratio of the right (treated) to left (untreated) tumor and expressed as a thermal enhancement ratio (TER).

The injection of PEGylated GNRs (60 × 15 nm ± 6 × 2 nm, SPR = 800 nm, Figure 1) in mice is well tolerated and no signs of distress or toxicity have been observed in this and other experiments. Immediately after initiation of laser treatment, temperatures inside the tumor climb rapidly and reach equilibrium within a few minutes (Figure 2). Though treatment with laser alone (absence of GNRs) does result in some tissue heating, the presence of GNRs significantly amplifies the degree of heat generation at both laser powers tested. The temperatures inside the tumors in the last 10 seconds of laser treatment were averaged and the changes in temperatures as well as final temperatures are listed in Table 1. When groups were treated with PPTT using a laser power equal to 1.6 W/cm^2 and 1.2 W/cm^2, the average equilibrium temperature inside the tumors reaches 46.3°C and 43.6°C respectively. Therefore, by changing the laser power alone, severe and moderate hyperthermia was able to be achieved.

After animal sacrifice 5 hours post laser treatment, the tumors were dissected out. In the animals receiving PPTT at 1.6 W/cm^2 significant bleeding was observed in most tumors due to conditions of severe hyperthermia. Additionally, the areas around the tumor were deeply colored in EBD indicating that the heat generated in the tumors caused the surrounding tissue to also heat. Though definitive conclusions cannot be made as to why this heating of normal tissue results in increased delivery of EBD, it is probable that the vessels dilated in response to insult and therefore the resulting increase in blood perfusion aided the delivery of EBD. In all other experimental groups, including animals treated with PPTT at 1.2 W/cm^2, no obvious hemorrhaging and local discoloration of surrounding tissue was observed.

Quantification of EBD in treated and untreated tumors, expressed as a ratio, indicates that PPTT does in fact enhance the delivery of macromolecules (Table 1 and Figure 3). When the average tumor temperature during PPTT was 46.3 and 43.6°C, the extravasation of EBD was enhanced 1.82 and 1.68-fold respectively. Though the TER is statistically different between groups with and without GNRs ($p<0.01$), no statistical difference is observed between both groups that received PPTT at different laser intensities. As expected, when laser treatment was applied without the presence of GNRs, the TER was around 1.0 indicating that the heat generated by laser alone, used under these study conditions, did not increase tumor microvascular permeability.

Hyperthermia enabled drug delivery has several limitations. There exists a very narrow window (roughly 42°C ≤ T ≤ 43°C) where increased blood perfusion and permeability is observed without severe vascular damage (Horsman, 2006). This is evidenced by the fact that in the present study, at 1.6 W/cm^2 without the presence of GNRs, the tumor temperature reached 41.2°C but did not result in any increased EBD delivery. Therefore, using standard techniques of inducing hyperthermia in the clinic, maintaining a tumor temperature within this therapeutic window is difficult. Also, non-specific heating of surrounding healthy tissue may increase the probability of drug delivery within those regions where undesired toxicity is likely to occur.

PPTT has the potential to partially address these issues. Control of laser beam power and alignment may enable clinicians to precisely control thermal dose in a directed way. Also, PPTT represents a targeted approach to hyperthermia. As GNRs primarily partition out of the blood due to EPR, it is unlikely that GNRs will reside in surrounding, healthy tissue. This provides a degree of safety if the laser beam were to radiate such tissue. This can be further improved with the attachment of targeting moieties such as peptides and antibodies. Unfortunately, because the heat distribution is dependent on GNR localization, a significant disadvantage is that tumor tissue without GNRs will not receive thermal therapy.

Using PPTT as a tool to induce hyperthermia and therefore increase the perfusion and permeability of tumor blood vessels may represent a new approach to augmenting macromolecular drug delivery. It has been shown that PPTT can be used to precisely control tumor temperature so that either moderate or severe tumor hyperthermia is obtained. This approach can therefore be used to increase the delivery of macromolecules such as albumin in the present paper. More detailed studies of resulting vascular events and delivery of macromolecular therapeutics for treatment of solid tumors is necessary.

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Abbreviations

EPR enhanced permeability and retention

EBD	Evans blue dye
GNR	gold nanorod
PPTT	plasmonic photothermal therapy
SPR	surface plasmon resonance
TER	thermal enhancement ratio
PEG	poly(ethylene glycol)
PBS	phosphate buffered saline

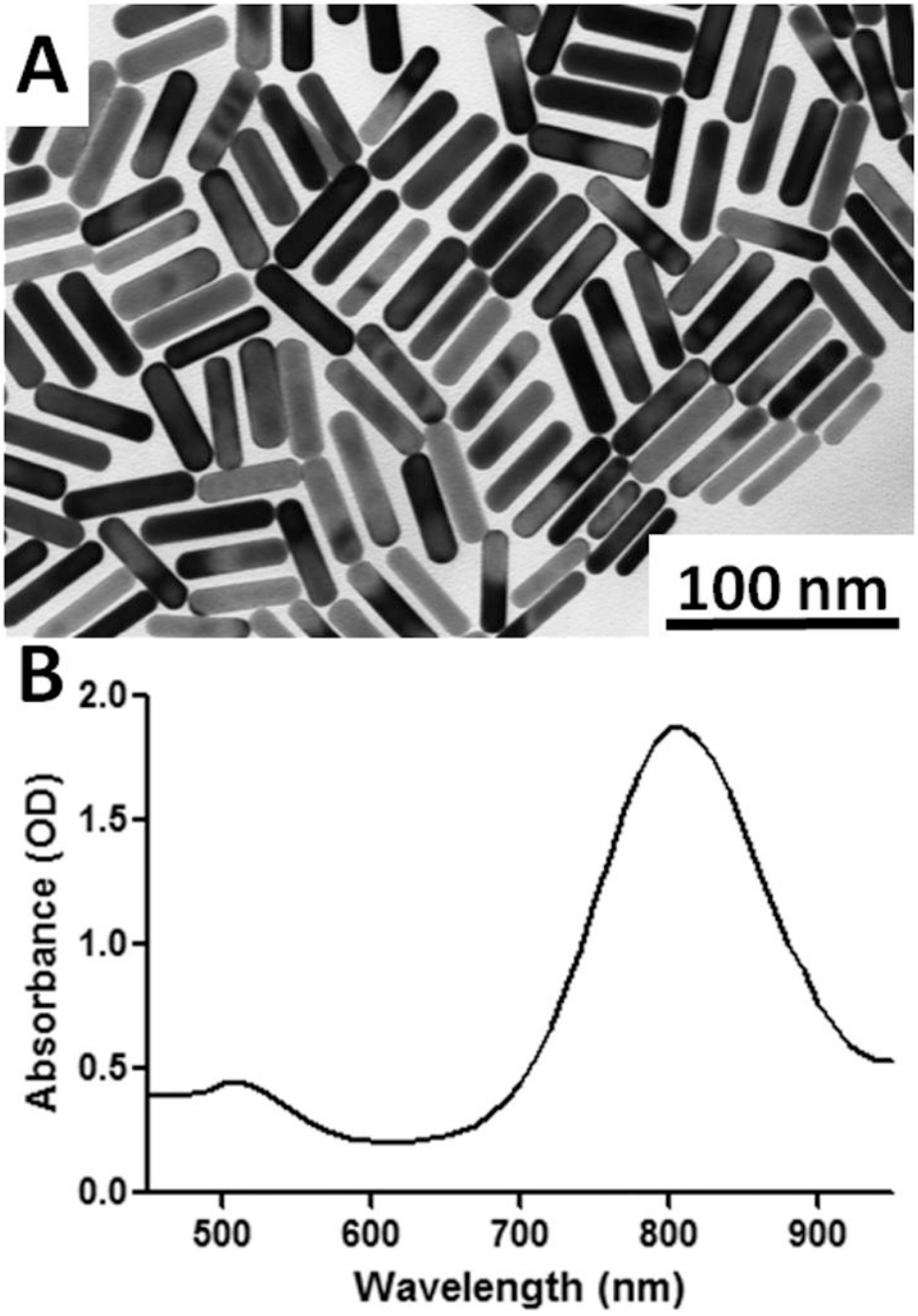

Figure 1.
Characterization of GNRs. A) Transmission electron micrograph of GNRs; and B) light absorption profile of GNRs with SPR peak at 800 nm.

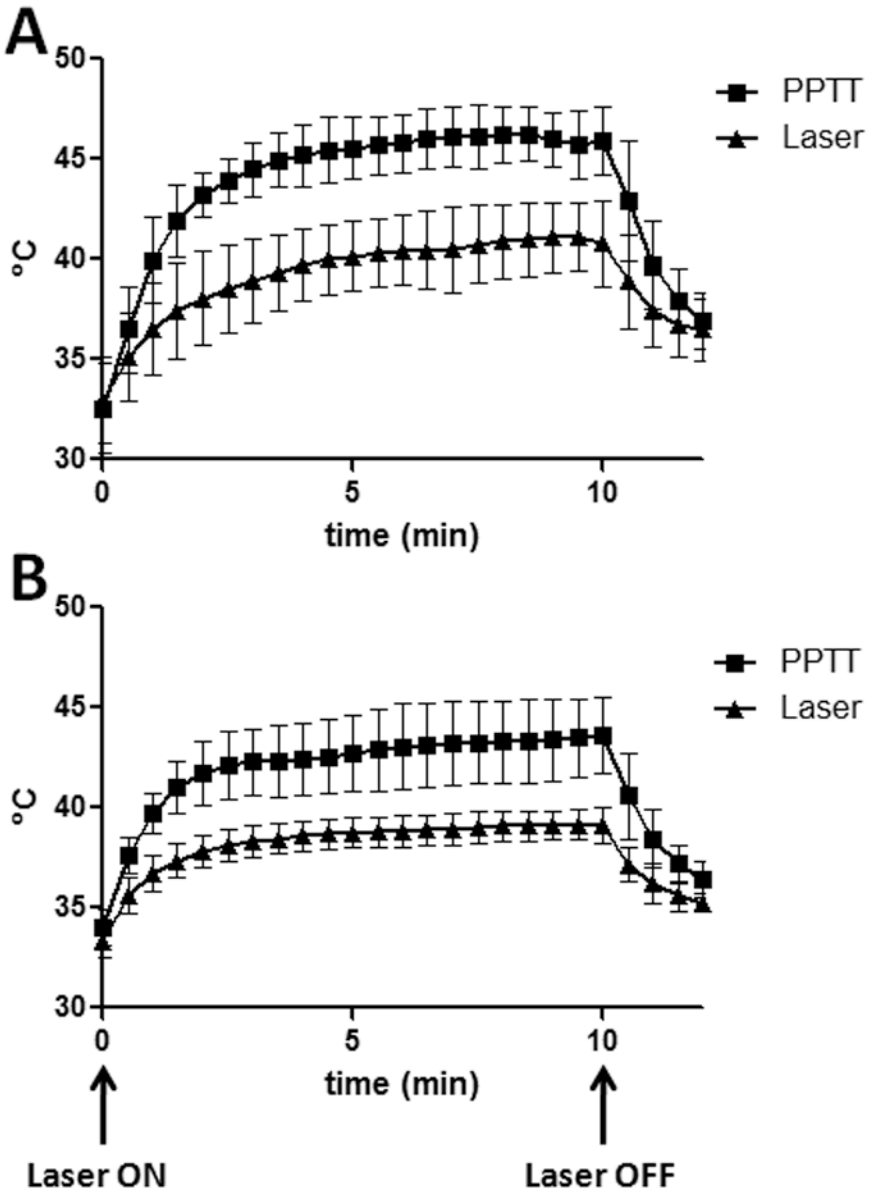

Figure 2.
Intratumoral temperatures during PPTT or laser alone. Laser power = 1.6 W/cm^2 (A) and 1.2 W/cm^2 (B). Error bars represented as ± standard deviation.

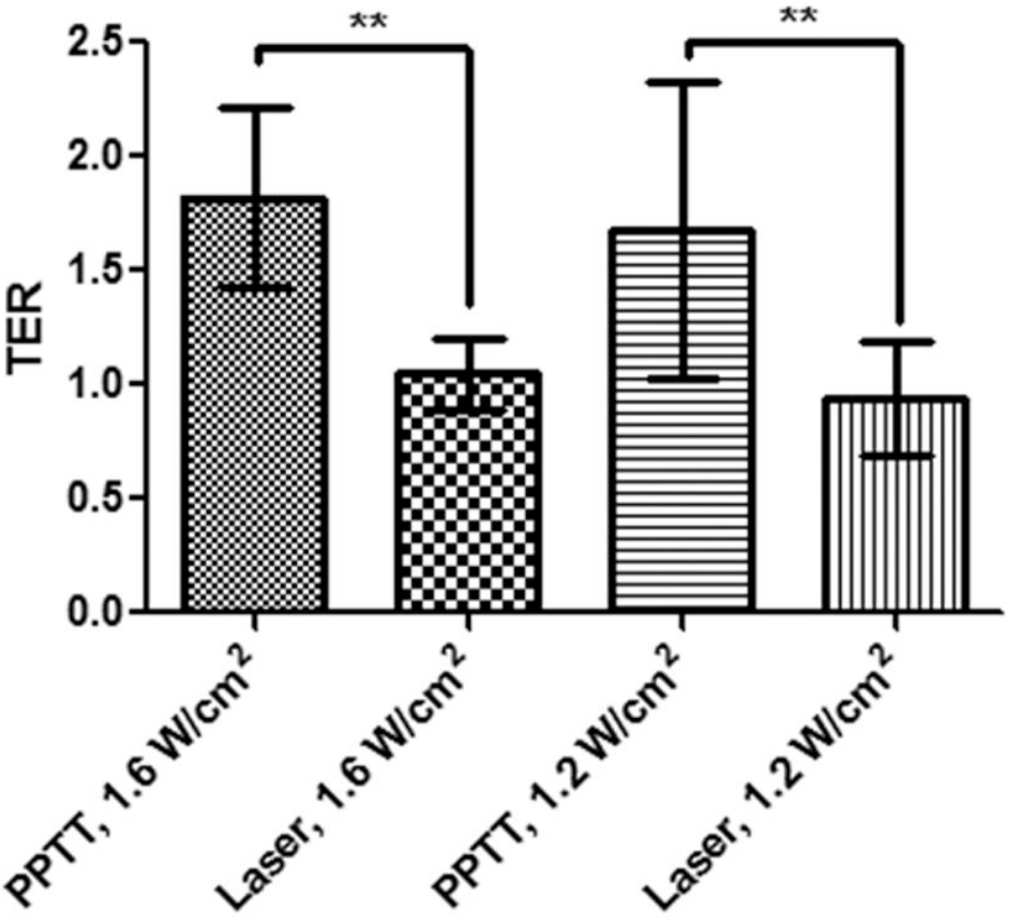

Figure 3.

Evans blue dye (EBD) delivery thermal enhancement ratio (TER). **Indicates a statistically significant difference ($p<0.01$) by one-way analysis of variance (ANOVA). Error bars represented as ± standard deviation.

Table I

Thermal Enhancement Ratio (TER)

Group	ΔT (°C)	Max T (°C)	TER
[a]PPTT, 1.6 W/cm^2	13.7 ± 2.9	46.3 ± 1.3	1.82 ± 0.40
[b]Laser, 1.6 W/cm^2	8.3 ± 1.8	41.2 ± 1.7	1.05 ± 0.15
[b]PPTT, 1.2 W/cm^2	9.6 ± 2.3	43.6 ± 1.9	1.68 ± 0.65
[c]Laser, 1.2 W/cm^2	6.0 ± 1.1	39.3 ± 0.8	0.94 ± 0.25

Numbers expressed as: mean ± standard deviation

[a] N = 7

[b] N = 6

[c] N = 10