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Imaging-guided high-efficient photoacoustic tumor therapy with targeting gold nanorods

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Abstract

Photoacoustic therapy using the large photoacoustic effect of agents for selectively killing cancer cells is demonstrated. Herein, a highly efficient photoacoustic treatment using gold nanorods (AuNRs) and its antitumor effect are reported. Folic acid conjugated AuNRs are designed to specifically target folate receptor-expressing cancer cells. Following photoacoustic treatment, most of the cancer cells with intracellular AuNRs die within 20 s. Compared with single-walled carbon nanotubes and indocyanine green containing nanoparticles, AuNRs can produce much stronger shock waves by absorbing the optical energy and thus induced the more efficient cell death at equal molar concentrations. In addition, the laser-induced shock waves can be detected for photoacoustic imaging. Our *in vivo* experiments demonstrated that the AuNR-mediated photoacoustic treatment resulted in efficient tumor suppression in mice. Thus, both efficient cancer cell diagnostics and selective photoacoustic treatment can be realized with a single-particle formulation.

Key words: Photoacoustic therapy; Photoacoustic imaging; Gold nanorods; Folic acid

Background

Among many cancer treatment methods, nanoparticle-mediated photoacoustic treatment is considered as one of the promising cancer treatment methods because of its non-invasiveness and cancer-specific treatment.[1-3] Photoacoustic treatment uses the photoacoustic effect of photoabsorbers to selectively destruct cancer cells. When the photoabsorbers are exposed to a pulsed laser beam, light energy can be transformed into acoustic energy. Then the acoustic waves will be generated. That is the so called photoacoustic effect. The magnitude of photoacoustic amplitude can generate a strong shock wave, with peak pressure of 100 Mpa.[4] Such a strong shock wave can result in the death of cell.[1-3] The laser-induced shock waves can also be detected by a highly sensitive ultrasound transducer, and thus used to spatially resolve the location of absorbers, namely photoacoustic imaging.[5-12] Therefore, combined photoacoustic imaging and photoacoustic therapy can be achieved with a single-particle formulation.

Photothermal therapy and high-intensity focused ultrasound (HIFU) are thermal tissue ablation methods. Both methods may be limited in their applicability to the treatment of tumors owing to the exposure to continuous laser irradiation or ultrasound, which may cause a 'heat sink' effect with thermal diffusion, resulting in the damage of adjacent normal cells.[13,14] In addition, gas in the bowel cannot be penetrated by HIFU, and sound waves are reflected back toward the transducer, which have high energy and may produce burns in the intervening tissue.[14] Recently, we introduce the method of photoacoustic therapy, based on mechanical, rather than thermal, destruction of individual target cells with laser-induced shock waves. Heat conduction is very low in the photoacoustic technique because the duration of the laser pulse (4-6 ns) is much shorter than the thermal diffusion time (submicroseconds).[15,16] Photoacoustic treatment has several

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potential advantages. First, the laser power used for cancer killing could be reduced 150-1500 times compared with other laser-thermal effect based techniques.[2] Second, mechanical damage of cell does not cause toxicity and drug resistance.[2] Third, these methods offer the required localized damage on tumor cells without damaging the surrounding tissues.[1] Last, the shock waves generated from cancer-specific photoabsorbers could be simultaneously used to image and treat cancers.

The key component of photoacoustic treatment is photoabsorbers that can absorb and convert light energy into acoustic energy with high efficiency. By now, two types of photoabsorbers have been reported for photoacoustic treatment (single-walled carbon nanotubes (SWNTs)[1,2] and indocyanine green containing nanoparticles (ICG-PL-PEG)[3]). Among the materials investigated for photoactivated imaging and therapy, AuNRs appear to be one of the most effective agents to date. AuNRs exhibit many unique intrinsic photophysical properties and have been intensively explored for biological and biomedical applications in the past several years. (1) AuNRs possess excellent surface plasma resonances in the near-infrared (NIR) that is readily tunable to regions where optical transmission through tissues is at its maximum.[17-19] (2) AuNRs exhibit about 160 times and 5000 times higher optical absorbance than SWNTs and ICG, respectively.[20,21] (3) AuNRs exhibit ease of synthesis and surface and structure modification.[18,19] (4) AuNRs can simultaneously be the imaging and therapeutic agents for combined diagnosis and therapy.[22-24] AuNRs have been used as photothermal agents[25-27] and photothermolysis agents[28-32] for killing cancer cells. Moreover, AuNRs have also been used as contrast agents for cancer imaging during pulsed laser irradiation.[33]

In the present study, the properties of AuNRs were compared with SWNTs and ICG-PL-PEG. The results showed that AuNRs exhibited the highest optical energy absorbance and produced the strongest photoacoustic intensity at the same molar concentration. Then AuNRs conjugated with FA as targeted absorbers were internalized into human epithelial carcinoma cells (HeLa). Following photoacoustic treatment, shock waves were produced *in situ* and the cancer cells were greatly destroyed for all these AuNRs. The shock waves generated from AuNRs were applied in combined photoacoustic treatment and imaging. Compared with SWNTs and ICG-PL-PEG, AuNR-mediated photoacoustic treatment induced the most efficient cancer cell destruction. *In vivo* pharmacokinetic studies showed that the FA-AuNRs had significant tumor accumulation in a mouse tumor model. Following photoacoustic treatment, the highly efficient destruction of solid tumors *in vivo* was confirmed. This work provided vital information to aiding the improvement of photoactivated treatment.

Methods

AuNR synthesis

AuNRs were prepared as described by Sau et al.[34] Gold seed particles were prepared by adding 250 μL of 10 mM hydrogen tetrachloroaurate($HAuCl_4 \cdot 3H_2O$) to 7.5 mL of 100 mM hexadecyltrimethylammonium bromide (CTAB) in a plastic tube with brief, gentle mixing. The solution appeared bright brown-yellow in color. Next, 600 μL of freshly prepared, ice-cold 10 mM $NaBH_4$ solution was added at once, followed by rapid inversion mixing for 2 min. The solution developed a pale brown-yellow color. The pale brown seed solution was stable and usable for several hours. For the growth of gold nanorods, 40 mL of 100 mM CTAB, 1.7 mL of 10 mM $HAuCl_4 \cdot 3H_2O$ and 250 μL of 10 mM $AgNO_3$ solution were added in that order, one by one, to a test tube, followed by gentle mixing. Then, 270 μL of 100 mM ascorbic acid was added, which changed the solution from brown-yellow to colorless. Finally, 420 μL of the seed solution was added to the growth solution, mixed gently, and left still for 3 h.

Functionalization of AuNRs and SWNTs

For polyethylene glycol (PEG) modification, amine-PEG-thiol (HS-PEG-NH_2) was added to a dispersion of the AuNRs. The raw AuNR solution was centrifuged at 7000 g for 20 min to eliminate the excess CTAB. After the colorless supernatant solution was gently removed, the pellets at the bottom of centrifuge tubes were redispersed by adding 5 mL of 2 mM HS-PEG-NH_2. The mixture was incubated for 12 h. Then, the PEGlyated AuNRs were again centrifuged to remove any unreacted HS-PEG-NH_2 series and redispersed by adding water or PBS solution.

FA solution was activated by EDC/NHS to afford FA-NHS (molar ratio, FA:EDC:NHS = 1:1:1). HS-PEG-FA was produced by incubating FA-NHS with HS-PEG-NH_2 (molar ratio, FA-NHS:HS-PEG-NH_2 = 2:1, pH 7.4) for 4 h. After reaction, the solution was filtrated using 2000 Da filters (Millipore) to remove excess FA-NHS, EDC and NHS.

For conjugation of FA on AuNRs, 5 mL of 2 mM HS-PEG-FA was added to the raw AuNR solution. For conjugation of FA and fluorescein isothiocyanate (FITC) on AuNRs, 2.5 mL of 2 mM HS-PEG-FA and 2.5 mL of 2 mM HS-PEG-FITC were added to the raw AuNR solution. The mixture was allowed to react overnight at room temperature with protection from light. Then, the solution was centrifuged to excess removal of unconjugated HS-PEG-FA and HS-PEG-FITC.

ICG-PL-PEG-FA were prepared as described by Zheng et al.[35,36] FA-SWNTs were prepared as described by Kang et al.[2]

Photoacoustic imaging system

Photoacoustic microscopy (PAM) system was described by Yang et al.[37] A focused ultrasound transducer with center frequency of 15 MHz and −6 dB bandwidth of 100% was used to receive the photoacoustic signals generated by the tested sample. Photoacoustic computed tomography (PAT) system was described by Yang et al.[38] In the PAT system, based upon a 10 MHz 384-element ring ultrasound array, the incident fluence levels were maintained below 10 mJ/cm^2. An optical parametric oscillator (OPO) (Surelite II-20, Continuum, Santa Clara, CA, USA) with pulse duration of 4-6 ns and pulse repetition rate of 20 Hz was used as the light source. The OPO operated at 800 nm, 808 nm or 1064 nm was used to irradiate the samples for generating photoacoustic signals.