Biomaterials 35 (2014) 8206–8214

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Near-infrared dye bound albumin with separated imaging and therapy wavelength channels for imaging-guided photothermal therapy

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ARTICLE INFO

Article history:
Received 15 April 2014
Accepted 4 June 2014
Available online 21 June 2014

Keywords:
NIR dye
Human serum albumin
Fluorescent imaging
Photothermal therapy
Tumor metastasis

ABSTRACT

Development of theranostic agent for imaging-guided photothermal therapy has been of great interest in the field of nanomedicine. However, if fluorescent imaging and photothermal ablation are conducted with the same wavelength of light, the requirements of the agent's quantum yield (QY) for imaging and therapy are controversial. In this work, our synthesized near-infrared dye, IR825, is bound with human serum albumin (HSA), forming a HSA-IR825 complex with greatly enhanced fluorescence under 600 nm excitation by as much as 100 folds compared to that of free IR825, together with a rather high absorbance but low fluorescence QY at 808 nm. Since high QY that is required for fluorescence imaging would result in reduced photothermal conversion efficiency, the unique optical behavior of HSA-IR825 enables imaging and photothermal therapy at separated wavelengths both with optimized performances. We thus use HSA-IR825 for imaging-guided photothermal therapy in an animal tumor model. As revealed by in vivo fluorescence imaging, HSA-IR825 upon intravenous injection shows high tumor uptake likely owing to the enhanced permeability and retention effect, together with low levels of retentions in other organs. While HSA is an abundant protein in human serum, IR825 is able to be excreted by renal excretion as evidenced by high-performance liquid chromatography (HPLC). In vivo tumor treatment experiment is finally carried out with HSA-IR825, achieving 100% of tumor ablation in mice using a rather low dose of IR825. Our work presents a safe, simple, yet imageable photothermal nanoprobe, promising for future clinical translation in cancer treatment.

1. Introduction

Photothermal therapy (PTT) is a hyperthermia therapeutic method that usually employs light absorbing agents to kill cancer cells under laser irradiation by heating with high specificity and minimal invasiveness [1,2]. An ideal photothermal agent should exhibit strong absorbance in the near-infrared (NIR) tissue transparency window, together with a low fluorescence quantum yield (QY), so as the absorbed light energy could be effectively converted into heat instead of fluorescence emission [3,4]. For safe and efficient PTT, the photothermal agents should be non-toxic, and show high tumor specific accumulation without too much retention in other normal organs [5,6]. Moreover, to improve the therapeutic efficacy and minimize side effects in PTT, imaging may be conducted to guide the design of therapy plans, such as to choose the optimal irradiation region and optical doses, as well as to decide the best timing of laser treatment when the photothermal agent reaches the peaked accumulation in the targeted lesion [7,8].

In the past decade, numerous types of nanomaterials with strong NIR absorbance have been developed as photothermal agents, showing encouraging therapeutic results in many preclinical animal experiments, in most of which cases subcutaneous tumor models have been used. A large variety of inorganic nanomaterials, such as gold nanostructures (nanoshells, nanorods and nanocages) [9–13], carbon nanomaterials (carbon nanotube and graphene) [14–16], palladium nanosheets [17,18], copper sulfide nanoparticles [19,20]and tungsten oxide nanowires [21]have been widely explored by many groups including ours as photothermal agents. Although many of the above materials have shown

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http://dx.doi.org/10.1016/j.biomaterials.2014.06.013
0142-9612/

high efficacy for cancer therapy in animal experiments, the non-biodegradable nature of most of these inorganic PTT agents have significantly hampered their clinical applications due to concerns regarding their potential long-term toxicity [22]. Recently, a number of organic conjugated polymers such as polyaniline and polypyrrole have also attracted significant attention as PTT agents [6,23–25]. In spite of the encouraging PTT therapeutic effects obtained by using those polymeric nanoparticles, the biodegradation behaviors of these conjugated polymers remain unclear. Other types of organic PTT agents, such as micelles and liposomes encapsulating NIR dyes (e.g. Indocyanine Green, ICG), porphysomes, as well as protein-based theranostic agents, may have less safety concerns, and could be used for both NIR fluorescence imaging and phototherapy under single wavelength excitation [26–31]. However, for those imageable agents using the same wavelength channel for imaging and therapy, while high fluorescent QY would reduce the photothermal conversion efficiency, a low QY is not ideal for fluorescence imaging. The requirements for optical therapy and imaging are thus controversial in those systems.

In this work, a NIR dye, IR825, is bound with human serum albumin (HSA), obtaining a HSA-IR825 complex with separated imaging and therapy wavelength channels for imaging-guided photothermal therapy. Interestingly, our HSA-IR825 complex shows greatly enhanced fluorescence under 600 nm excitation with a high QY at ~42%, useful for in vivo fluorescence imaging. In the meanwhile, HSA-IR825 also exhibits another rather high absorbance peak at ~820 nm, under excitation by which wavelength the fluorescence QY is as low as ~0.33%, ideal for effective photothermal conversion in PTT cancer treatment. In our animal experiments, by fluorescence imaging with 600-nm excitation, we uncover that HSA-IR825 after intravenous injection shows highly efficient passive tumor accumulation in subcutaneous tumors, which could be effectively ablated under irradiation by an 808-nm laser. Considering the easy renal excretion of IR825 after systemic administration of HSA-IR825, and the inherent biocompatibility of HSA, an abundant human protein, our HSA-IR825 formulation may indeed be promising for future clinical use.

2. Experimental section

2.1. Materials

Human serum albumin (HSA) was purchased from Sigma–Aldrich. Bovine serum albumin (BSA) was purchased from J&C chemical CO. Methanol (CH_3OH) and triethylamine (TEA) were purchased from Sinopharm Chemical Reagent Co. IR825 dye was synthesized following our previously reported protocol [4].

2.2. Synthesis of HSA-IR825 nanocomplex

Our previous study has found that IR825 exhibited strong binding to proteins coated on the nanoparticle surface [32]. However, no enhancement of IR825 fluorescence was observed in that case because we loaded too many IR825 molecules onto nanoparticles, leading to the self-quenching as we also found in the current work (Supporting Fig. S5). In this study, to prepare HSA-IR825 complex, 5 mg as-synthesized IR825 was firstly dispersed in 1 ml methanol containing 10 μL TEA. 300 μL (1 eq) above-mentioned mixture was added into 100 mg (1 eq) HSA dispersed in 10 ml water. The HSA-IR825 nanocomplexes were obtained after dialyzing the solution for 8 h in a dialysis bag (MWCO 3500 Da). The encapsulation efficiency of IR825 by HSA was nearly 100%, as evidenced by the fact that the filtrate was completely colorless even after a long period of dialysis.

2.3. Characterization

Fluorescence spectra of different samples were obtained on a FluoroMax 4 luminescence spectrometer (HORIBA Jobin Yvon). UV-vis-NIR absorbance spectra were recorded by using a PerkinElmer Lambda 750 UV-vis-NIR spectrophotometer. The dynamic diameter of HSA-IR825 was determined by a Zetasizer Nano-ZS (Malvern Instruments, UK).

The quantum yield (QY) of HSA-IR825 under 600 nm and 808 nm laser excitation was estimated using rhodamine B and ICG as the standard references. The equation to calculate the sample quantum yields is provided as shown in Equation (1):

$$QY_U = QY_S \times \frac{F_U}{F_S} \times \frac{A_S}{A_U} \quad (1)$$

QY_U and QY_S are the absolute quantum yields of HSA-IR825 and the standard reference, respectively. F_U and F_S are the integrated fluorescence intensities of HSA-IR825 and rhodamine B/ICG., respectively. A_U and A_S are the absorption values of the two samples at this excitation wavelength. Using this method, the QY of HSA-IR825 at 600 nm and 808 nm was measured to be 42.1% and 0.33%, respectively.

2.4. Binding structure simulation

The crystal structure of human serum albumin (HSA, PDB entry: 4K2C) was retrieved from Protein Data Bank (http://www.pdb.org/pdb/) and then prepared by Discovery Studio 2.5 (Discovery Studio, version 2.5, 2009, Accelrys Inc., San Diego, CA, USA.), including residues repair and energy minimization. We used CDocker program in Discovery Studio 2.5 to construct the 3D models of HSA and IR825. The dock program CDocker and DS catalyst Score are applied to construct receptor–ligand complexes. The binding site of the receptor is set at the active site of HSA with a radius of 5 Å, large enough to cover the binding pocket. CDocker is a grid-based molecular docking method that employs CHARMM forcefield. The receptor is held rigid while the ligands are allowed to flex during the refinement. For pre-docked ligands, prior knowledge of the binding site is not required. It is possible, however, to specify the ligand placement in the active site using a binding site sphere. Random ligand conformations are generated from the initial ligand structure through high temperature molecular dynamics, followed by random rotations. The random conformations are refined by grid-based simulated annealing and a final grid-based or full forcefield minimization.

2.5. Determination of IR825 concentrations in urine samples by high-performance liquid chromatography (HPLC)

Urine samples were collected from either untreated mice or mice i.v. injected with HSA-IR825 at different time points. Each sample was dissolved in 200 μL lysis buffer (1% SDS, 1%Triton X-100, 40 mM Tris Acetate) and 1.8 ml methanol. After incubation at room temperature for 12 h, the samples were centrifuged at 24,000 g for 10 min to remove aggregates. The amount of IR825 was determined by a HPLC (Agilent 1260) with a UV-vis detector at 227 nm. A mixture of methanol and water at 1:1 (v/v) was used as the mobile phase. The elution peak of IR825 was found to be at 8.8 min.

2.6. Cellular experiments

4T1 murine breast cancer line was originally obtained from American Type Culture Collection (ATCC) and cultured under recommended conditions. The *in vitro* cytotoxicity was measured using a standard methyl thiazolyltetrazolium (MTT, Sigmae Aldrich) assay. For cell toxicity assay, 4T1 cells were seeded into 96-well cell culture plates at 1×10^5/well until adherent and then incubated with various concentrations of HSA-IR825 for 24 h. The standard MTT assay was carried out to determine the cell viabilities relative to control untreated cells. For photothermal therapy, 4T1 cells were seeded into 96-well plates at 1×10^5/well until adherent and then incubated with 4 μM HSA-IR825 for ~4 h. Then the cells were irradiated by an 808 nm laser at different power densities (0, 0.5, 0.8, 1.0, 1.2 W/cm^2) for 10 min before the MTT assay. In addition, 4T1 cells were incubated with different concentrations of HSA-IR825 and then irradiated by an 808 nm laser at power densities of 1 W/cm^2. The cells were stained with Calcine AM/PI for 30 min, washed with PBS, and then imaged by a confocal fluorescence microscope.

For confocal fluorescence imaging, 4T1 cells (1×10^5 cells) were cultured in 35 mm culture dishes containing 4 μM HSA-IR825 for different times. After washing with PBS (pH = 7.4) for three times, cells were labeled with 4′, 6-diamidino-2-phenylindole (DAPI) and then imaged by a laser scanning confocal fluorescence microscope (Leica SP5), to determine the cellular uptake of HSA-IR825.

2.7. Subcutaneous tumor model for imaging and therapy

Female nude mice and Balb/c mice were purchased from Nanjing Peng Sheng Biological Technology Co Ltd and used under protocols approved by Soochow University Laboratory Animal Center. 1×10^6 4T1 cells suspended in 40 μL phosphate buffered saline (PBS) were subcutaneously injected into the back of each female nude mouse. After ~7 days, the mice bearing 4T1 tumors were intravenously (i.v.) injected 0.15 μmol HSA-IR825 (200 μL 50 mg/ml HSA, 0.13 mg/ml IR825) when the tumor volume reached ~60 mm^3. All images were analyzed and collected at the indicated time point with a Maestro in vivo optical imaging system (Cambridge Research & instrumentation, Inc). The mice were sacrificed at 2 h after i.v. injection, with their major organs including the tumor, liver, heart, lung, spleen, and kidneys collected and imaged by the in vivo imaging system.

For in vivo photothermal therapy, nude mice bearing subcutaneous 4T1 tumors (~60 mm^3) were divided into four groups (n = 5 per group): (a) untreated; (b) irradiated by the 808 nm laser (0.8 W/cm^2, 10 min) only; (c) i.v. injected with 200 μl HSA-IR825 (50 mg/ml HSA, 0.13 mg/ml IR825) only; (d) i.v. injected with 200 μl HSA-IR825 (0.75 μmol/ml) and irradiated by the 808-nm laser (0.8 W/cm^2, 10 min, 2 h after injection). An IR thermal camera (Infrared Cameras. Inc) was used to monitor