Journal of Controlled Release 241 (2016) 186–193

Contents lists available at ScienceDirect

Journal of Controlled Release

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

High intensity focused ultrasound hyperthermia for enhanced macromolecular delivery

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ARTICLE INFO

Article history:
Received 17 March 2016
Received in revised form 11 September 2016
Accepted 24 September 2016
Available online 27 September 2016

Keywords:
Macromolecular delivery
High intensity focused ultrasound
Hyperthermia
Drug delivery
Magnetic resonance imaging

ABSTRACT

Mild hyperthermia has been used in combination with polymer therapeutics to further increase delivery to solid tumors and enhance efficacy. An attractive method for generating heat is through non-invasive high intensity focused ultrasound (HIFU). HIFU is often used for ablative therapies and must be adapted to produce uniform mild hyperthermia in a solid tumor. In this work a magnetic resonance imaging guided HIFU (MRgHIFU) controlled feedback system was developed to produce a spatially uniform 43 °C heating pattern in a subcutaneous mouse tumor. MRgHIFU was employed to create hyperthermic conditions that enhance macromolecular delivery. Using a mouse model with two subcutaneous tumors, it was demonstrated that MRgHIFU enhanced delivery of both Evans blue dye (EBD) and Gadolinium-chelated *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers. The EBD accumulation in the heated tumors increased by nearly 2-fold compared to unheated tumors. The Gadolinium-chelated HPMA copolymers also showed significant enhancement in accumulation over control as evaluated through MRI T1-mapping measurements. Results show the potential of HIFU-mediated hyperthermia for enhanced delivery of polymer therapeutics.

1. Introduction

Many conventional chemotherapeutics exhibit <1% accumulation of the injected dose (ID) within solid tumors [1]. This can be attributed to poor water-solubility, short circulation half-life, and biological barriers hindering extravasation and penetration [2]. To overcome these issues, nanomedicines have been developed to increase site-specific accumulation through passive targeting by the enhanced permeability and retention (EPR) effect and through active targeting strategies to ultimately improve efficacy. Although improvements have been observed with these systems, the increased accumulation is marginal at best where <5% ID localizes within the tumor [1]. Ways to further enhance the delivery of nanomedicines include augmentation of the EPR effect with the use of vascular mediators or mild hyperthermia to increase blood flow and vascular permeability [3].

Hyperthermia has been used as a combination therapy to further increase the delivery of targeted nanomedicines and enhance efficacy of chemotherapy [4]. This is accomplished by increasing blood flow in the heated tumor tissue while also dilating the tumor vessels, further expanding the fenestrae and allowing for greater extravasation. Methods to selectively heat the tumor tissue include radiofrequency ablation (RFA), magnetic fluid hyperthermia (MFH), gold nanoparticle mediated laser therapy, and high intensity focused ultrasound (HIFU). Previously, our lab utilized gold nanorod (GNR)-mediated plasmonic photothermal therapy (PPTT) to enhance the delivery of water-soluble HPMA copolymers. This method was shown to be effective in improving delivery [5], tumor penetration [6], and efficacy [7] against prostate cancer xenografts. However, the application of heat through this method is limited by several factors. Delivery of gold nanoparticles to the tumor site mainly depends on the EPR effect which may not be exhibited in all tumors and tumor types [2], and can limit the heating capacity in methods that require nanoparticle accumulation by this route. Systemic administration of gold

Abbreviations: (AIBN), Azobisisobutyronitrile; (APMA-DOTA), aminopropylmethacrylamide-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; (DI), deionized; (EBD), Evans blue dye; (EDTA), ethylenediaminetetraacetic acid; (EPR), enhanced permeability and retention; (FDA), Food and Drug Administration; (Gd), Gadolinium; (GNR), gold nanorod; (GRP78), glucose regulated protein-78; (HIFU), high intensity focused ultrasound; (HPMA), *N*-(2-hydroxypropyl)methacrylamide; (ICP-MS), inductively coupled plasma mass spectrometry; (ID), injected dose; (MFH), magnetic fluid hyperthermia; (M_n), number average molecular weight; (MRI), Magnetic resonance imaging; (M_w), Weight average molecular weight; (M_w/M_n), Polydispersity index; (MRgHIFU), magnetic resonance imaging-guided HIFU; (PPTT), plasmonic photothermal therapy; (RFA), radiofrequency ablation; (ROI), region of interest; (SEC), size exclusion chromatography; (Seg-EPI), segmented-echo planar imaging; (TE), echo time; (TER), thermal enhancement ratio; (TR), repetition time.

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http://dx.doi.org/10.1016/j.jconrel.2016.09.030
0168-3659/

nanoparticles leads to long-term accumulation in filtration organs, such as the liver and spleen, as a majority of the GNRs accumulate in these tissues (>90%) as opposed to the tumor tissue [8]. The potential adverse effects over time of this off-target accumulation are not fully understood, potentially hampering their translation to the clinic. Additionally, limited penetration depth of light reduces the utility of GNR-mediated mild hyperthermia to superficial tumors. Alternative methods are needed to generate mild hyperthermia to enhance delivery of nanomedicines.

One such method is high intensity focused ultrasound (HIFU). This method generates heat without the insertion of a probe as with RFA or prior injection of nanoparticles as with MFH or PPTT. HIFU produces heat within the body through focusing ultrasound waves to a focal point creating an intense deposition of energy that can cause change at the cellular level using both thermal and mechanical effects. This phenomenon can cause heating in tissues resulting in hyperthermic or ablative effects. Using phased-array transducers with multiple transducer elements, the focal point can be electronically phased to compensate for the acoustic properties of different tissues and create subject-specific heating patterns [9]. HIFU additionally allows for heating with a high degree of temporal control, as the rate of heating depends on the magnitude and duration of the ultrasound exposure [10]. While HIFU has been performed under ultrasound and magnetic resonance imaging (MRI) guidance, MRI provides excellent soft tissue contrast as well as the ability to monitor the temperature rise in real-time through MRI thermometry. Integration of MR guidance during HIFU exposure allows for simultaneous imaging to guide the treatment and MR thermometry to monitor the temperature and provide real-time feedback [11,12].

One challenge of HIFU in comparison to the other methods of heating is that focal zone does not preferentially heat the tumor tissue and needs to be guided to the tumor site through MR or ultrasound imaging prior to treatment. The HIFU focal spot also has a relatively small, ellipsoidal focal zone, on the scale of a few millimeters (e.g. 1–3 × 3–8 mm), and needs to be moved throughout a larger tumor to achieve uniform heating [13]. Electronic beam steering or physically steering the transducer can be used in combination with real-time temperature mapping by MR thermometry to heat the pathological tissues at a predefined temperature over a certain length of time [14]. This MR-guided HIFU (MRgHIFU) technology platform is currently Food and Drug Administration (FDA) approved for thermal ablation of uterine fibroids, treatment of bone metastases, and for treatment of prostate cancer [15], but has potential applications in temperature-induced local drug delivery with hyperthermia [16] and other ablative therapies in oncology (i.e. breast, prostate, liver, brain) [17]. Because of these attributes, HIFU has emerged as an effective modality for drug delivery applications.

The aim of this work was to create an MRgHIFU controller system to uniformly heat tumor tissue in a subcutaneous mouse tumor model at approximately 43 °C and further enhance the delivery of macromolecules. Ex vivo techniques were first used to evaluate the MRgHIFU controller system and determine treatment parameters that would achieve uniform hyperthermia. These parameters were then translated to the in vivo model. The ability to enhance delivery of macromolecules including Evans blue dye (EBD) bound to albumin and HPMA copolymers was evaluated.

2. Materials and methods

2.1. Synthesis and characterization of HPMA copolymers

HPMA [18] and aminopropylmethacrylamide-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (APMA-DOTA) [19] comonomers were synthesized as described previously. Free radical precipitation copolymerization using Azobisisobutyronitrile (AIBN) as the initiator in methanol at 50 °C for 24 h was used to prepare the copolymers. The product was then precipitated and washed in diethyl ether followed by dialysis against deionized water to remove unreacted comonomers and initiator. The copolymers were lyophilized to obtain the final product. Weight average molecular weight (M_w), number average molecular weight (M_n), and polydispersity index (M_w/M_n) were estimated by size exclusion chromatography (SEC). APMA-DOTA comonomer was included in copolymerization for the ability to chelate Gadolinium (Gd).

The resulting copolymer was then dissolved in deionized (DI) water with Gd (III) acetate hydrate (1.2 mol equivalent to APMA-DOTA) and the pH was raised between 5.0 and 5.5. The solution was stirred overnight for 16 h followed by addition of ethylenediaminetetraacetic acid (EDTA) to remove excess Gd (EDTA:GD, 1:1). The product was then dialyzed against 0.9% saline and lyophilized. M_w, M_n, and M_w/M_n were estimated by SEC. The amount of chelated Gd and free Gd was determined by inductively coupled plasma mass spectrometry (ICP-MS). Chelated Gd and free Gd were separated using a disposable size exclusion PD10 column and the different fractions were analyzed by ICP-MS to determine purity.

The longitudinal relaxivity (T1) of the copolymers was characterized and compared to previously synthesized Gd-chelated copolymers [6,19,20]. Four different concentrations of Gd (0.1 to 0.015 mM Gd) were prepared in DI water and placed in a Bruker BioSpec 7.1 T horizontal-bore MRI. T1 values were measured by an inversion recovery fast spin-echo imaging sequence using inversion times of 50, 100, 300, 500, 800, 1000, 2000, 4000, 7000 and 8000 ms, echo time (TE) of 4.2 ms, and repetition time (TR) of 12,000 ms. T1 for each vial was calculated using Bruker software and the relaxation rate (R1 = 1/T1) was plotted against Gd equivalent concentration. The relaxivity was measured as the slope of this plot.

2.2. Stability of HPMA copolymer-Gd conjugates

HPMA copolymer-Gd conjugates were dissolved in mouse serum and incubated at 43 °C for 10 min followed by incubation at 37 °C for a total of 72 h. Samples were analyzed at 10 min, 24, 36, and 72 h and run on a PD10 column to separate free Gd from HPMA copolymer-Gd conjugates. The fractions were then analyzed by ICP-MS for Gd content. The amount of free Gd was compared to that of the chelated HPMA copolymer-Gd fraction to determine the percent of free Gd over time.

2.3. MRgHIFU controller system and ex vivo evaluation

All heating was performed using an MRgHIFU small animal system (Image Guided Therapy, Inc., Bordeaux, France. 16-element annular transducer, aperture size of the transducer = 45 mm, f = 3 MHz, 1x1x3 mm full-width-half-maximum focal spot size, ±1.5 cm focusing along beam direction) placed in a Siemens 3 T Trio MRI scanner. Because the phased-array transducer has an annular design, in plane focal spot motion was achieved through physically moving the transducer through the use of piezoelectric motors. The experimental setup is shown in Fig. 1A. To determine MRgHIFU heating parameters that would produce stable hyperthermic conditions, different combinations of ultrasound power, heating trajectory shape, and speed were evaluated in an ex vivo chicken breast model. The transducer was initially calibrated by determining the pressure pattern using hydrophone scanning and the transducer efficiency was determined using a radiation force balance technique [21]. The applied power levels were determined in the ex vivo chicken breast model. To best mimic the conditions required for the in vivo model, uniformity of heating over a 10 × 10 mm region of interest (ROI) was evaluated for each parameter set, and the combination of parameters that produced a spatially uniform and stable temperature rise of 43 °C over a 10-minute period were identified. The transducer was moved continuously so there were no discrete step size issues with the motors. Temperatures were assessed in real-time using the proton resonance frequency shift MR thermometry method [22] using a 2D segmented-echo planar imaging (Seg-EPI) sequence (TR/TE = 150/13 ms, ETL = 9, 1.2 s acquisition, 2 × 2 × 3 mm resolution, 3 slices). Susceptibility effects due to ultrasound transducer motion were mitigated using an atlas-based reconstruction [23] where approximately 50 baseline library images were acquired with the transducer moving along the defined trajectory (Fig. 1B) multiple times without firing the ultrasound. During sonication,