



● Original Contribution

ENHANCEMENT OF SMALL MOLECULE DELIVERY BY PULSED HIGH-INTENSITY FOCUSED ULTRASOUND: A PARAMETER EXPLORATION

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(Received 17 February 2015; revised 9 December 2015; in final form 11 December 2015)

Abstract—Chemotherapeutic drug delivery is often ineffective within solid tumors, but increasing the drug dose would result in systemic toxicity. The use of high-intensity focused ultrasound (HIFU) has the potential to enhance penetration of small molecules. However, operation parameters need to be optimized before the use of chemotherapeutic drugs *in vivo* and translation to clinical trials. In this study, the effects of pulsed HIFU (pHIFU) parameters (spatial-average pulse-average intensity, duty factor and pulse repetition frequency) on the penetration as well as content of small molecules were evaluated in *ex vivo* porcine kidneys. Specific HIFU parameters resulted in more than 40 times greater Evans blue content and 3.5 times the penetration depth compared with untreated samples. When selected parameters were applied to porcine kidneys *in vivo*, a 2.3-fold increase in concentration was obtained after a 2-min exposure to pHIFU. Pulsed HIFU has been found to be an effective modality to enhance both the concentration and penetration depth of small molecules in tissue using the optimized HIFU parameters. Although, performed in normal tissue, this study has the promise of translation into tumor tissue. (E-mail: ynwang@u.washington.edu) © 2016 World Federation for Ultrasound in Medicine & Biology.

Key Words: Pulsed-high intensity focused ultrasound, Drug delivery, Content and penetration, Ultrasound parameters, Kidney.

INTRODUCTION

Globally, cancer accounts for nearly 1 in 8 deaths, more than HIV/AIDS, tuberculosis and malaria combined (Jemal et al. 2010). It is currently the second most common cause of death (behind cardiac disease) in the United States and countries of the European Union (Ferlay et al. 2010). According to the Surveillance, Epidemiology, and End Results (SEER) study and mortality data from the National Center for Health Statistics (NCHS), it is estimated that within the United States alone, there were approximately 1,658,370 new cancer cases diagnosed and about 589,430 cancer deaths in 2015 (American Cancer Society 2015).

Once diagnosed, numerous conventional and novel cancer therapies are used, including surgery, radiotherapy, chemotherapy, immunotherapy, gene therapy, hyperthermia and phototherapy. However, all of these therapies have been slow, if not ineffective, in dramatically improving survival rates. Chemotherapeutics are often the treatment of choice, but conventional systemic administration results in saturation of normal tissue and non-selective cytotoxicity. For chemotherapeutics to be effective, there must be direct contact between the drug and the cancer cell with sufficient dose. However, increasing the drug dose would significantly increase the toxicity to healthy tissues. Although encapsulation of anti-cancer agents can mitigate the side effects accompanied by systemic delivery, a technique for controlled delivery is still required to release them to the cancerous tissue.

The efficacy and tolerability of cancer chemo- and biotherapies are limited mainly because of poor

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penetration of anti-cancer drugs from the blood into tumor cells. The first barrier the drug must overcome is the vessel wall. Solid tumors have a high vascular density, but the blood vessels have anatomic and pathophysiologic abnormalities, such as large gaps between endothelial cells. As a result, tumor tissues exhibit selective extravasation and retention of macromolecular drugs, called the enhanced permeability and retention (EPR) effect (Fang et al. 2011). Once in the interstitium, molecule diffusion and convection can be slow because of high interstitial fluid pressure (IFP), as well as the presence of a prominent stromal matrix that separates the atypical vasculature from the tumor cells (Brown et al. 2003; Netti et al. 2000). Once at the cells, the last physiologic barrier for anti-cancer drugs is the cell membrane. There is a reservoir of clonogenic cells in the region between the maximum penetration of the drug and the onset of hypoxia, which can repopulate between sessions of chemotherapy. Therefore, strategies that enhance drug penetration have considerable potential to decrease cell viability. Anti-vascular endothelial growth factor treatment has been found to render vessels more functional, resulting in a decrease in IFP and a subsequent improvement of drug diffusion within solid tumors (Jain 2005). The extracellular matrix can also be modified by hyaluronidase treatment to enable drug penetration to distal cell layers (Kerbel et al. 1996; St. Croix et al. 1998) or pHIFU (Li et al. 2015). Modification of the pH of acidic organelles can reduce the sequestering effect for more penetration of tumor tissue and toxic effects in the cell nucleus (Ouar et al. 2003).

The use of ultrasound energy to enhance the efficiency of chemotherapy of tumors began as early as the 1970s (Frenkel 2008; Zhou 2013). The cytotoxic effect of nitrogen mustard on mouse leukemia L1210 cells after sonication, without any mechanical damage to cells, was observed (Kremkau et al. 1976). Tumors implanted into the hamster flank and sonicated with the co-administration of a chemotherapeutic (BCNU) improved the survival rate from 29.4% (sonication alone) to 40% (Fry and Johnson 1978).

Despite the promise of HIFU-enhanced drug delivery, the underlying mechanism is not fully understood and the parameters have yet to be optimized (Frenkel 2008; Mo et al. 2012). Most importantly, there are few studies on the role of HIFU in overcoming the interstitial barrier (Li et al. 2015). Current investigations are focused either on transportation of the drug to the cancer cell through membrane or delivery to the xenografted tumor, which does not have the dense stromal structure of the naturally formed one (Primeau et al. 2005). The absence of a dense stromal matrix in the implanted tumor model may be the reason for discrepancies in anti-cancer agent outcomes between animal experiments and clinical trials.

In this study, pHIFU was applied to enhance the penetration of small molecules into *ex vivo* tissue to explore a new strategy of overcoming the interstitial barrier. Both the mean arithmetical penetration depth and content of Evans blue albumin (EBA) were used to evaluate small molecule delivery efficiency through the capsule of porcine kidney samples. Ultrasound parameters such as spatial-average pulse-average acoustic intensity (I_{SAPA}), duty factor (DF) and pulse repetition frequency (PRF) were varied to optimize the treatment conditions and investigate the underlying mechanism. Furthermore, the pHIFU-enhanced penetration and concentration of EBA in porcine kidneys after intravenous injection were also confirmed *in vivo*.

METHODS

Experimental protocol

All experiments were performed with a custom-made air-backed concave HIFU transducer with a diameter of 34.9 mm, focal length of 50 mm and resonant frequency of 1.16 MHz (Hwang et al. 2005). The driving electronics consisted of a function generator (33120A, Agilent Technologies, Palo Alto, CA, USA), radiofrequency power amplifier (AP-400 B, ENI, Rochester, NY, USA) and custom-built impedance matching unit. A fiberoptic probe hydrophone (FOPH-2000, RP Acoustics, Leutenbach, Germany) was mounted to a three-dimensional translation stage (minimum step size of 5 μ m, BiSlide, Velmex, Bloomfield, NY, USA), immersed in a testing tank filled with degassed water, and used to determine the characteristics of the transducer beam (-6 -dB focal size of 4.1×12.6 mm, width $\times$ length). The acoustic power generated by the HIFU transducer was measured using a radiation force balance with an absorptive tile (Maruvada et al. 2007), and the spatial-average pulse-average acoustic intensity, I_{SAPA} , was calculated (Table 1) (Schafer and Lewin 1988). The acoustic parameters used in this study are listed in Table 2. A custom-built truncated cone was attached to the transducer and filled with degassed water to couple the acoustic wave propagation. The tip of the

Table 1. Measured peak positive and peak negative pressures at the focus and spatial-average pulse-average acoustic intensity of the HIFU transducer at varying output levels

I_{SAPA} (W/cm ²)	$ p^- $ (MPa)	p^+ (MPa)
705	3.68	10.72
800	4.21	13.21
870	5.96	16.89
940	6.79	19.23

HIFU = high-intensity focused ultrasound; I_{SAPA} = spatial-average pulse-average acoustic intensity.

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