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● *Original Contribution*

ULTRASOUND-STIMULATED DRUG DELIVERY FOR TREATMENT OF RESIDUAL DISEASE AFTER INCOMPLETE RESECTION OF HEAD AND NECK CANCER

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Abstract—Microbubbles triggered with localized ultrasound (US) can improve tumor drug delivery and retention. Termed US-stimulated drug delivery, this strategy was applied to head and neck cancer (HNC) in a post-surgical tumor resection model. Luciferase-positive HNC squamous cell carcinoma (SCC) was implanted in the flanks of nude athymic mice (N = 24) that underwent various degrees of surgical tumor resection (0%, 50% or 100%). After surgery, animals received adjuvant therapy with cetuximab-IRDye alone, or cetuximab-IRDye in combination with US-stimulated drug delivery or saline injections (control) on days 4, 7 and 10. Tumor drug delivery was assessed on days 0, 4, 7, 10, 14 and 17 with an *in vivo* fluorescence imaging system, and tumor viability was evaluated at the same times with *in vivo* bioluminescence imaging. Tumor caliper measurements occurred two times per week for 24 d. Optical imaging revealed that in the 50% tumor resection group, US-stimulated drug delivery resulted in a significant increase in cetuximab delivery compared with administration of drug alone on day 10 (day of peak fluorescence) ($p = 0.03$). Tumor viability decreased in all groups that received cetuximab-IRDye in combination with US-stimulated drug delivery, compared with the group that received only the drug. After various degrees of surgical resection, this novel study reports positive improvements in drug uptake in the residual cancer cells when drug delivery is stimulated with US. (E-mail: hoyt@uab.edu) © **2014 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Adjuvant therapy, Cetuximab, Drug delivery, Head and neck cancer, Microbubbles, Optical imaging, Ultrasound.**

INTRODUCTION

Tumor retention and vascular permeability can be reversibly enhanced using mechanically oscillating microbubble (MB) contrast agents exposed to a low-intensity ultrasound (US) field in a process known as US-stimulated drug delivery. This strategy was first introduced to augment delivery of molecules through the blood-brain barrier (Hynynen et al. 2003; Marty et al. 2012; McDannold et al. 2012; Schlachetzki et al. 2002), and has since been investigated for multiple other purposes. This transient increase in membrane permeability introduces a therapeutic window in which enhanced drug uptake occurs, thereby improving the anti-cancer effects. This relatively non-invasive approach to cancer treatment is generally considered non-toxic, tolerable and effective. US-stimulated drug delivery has been reported to result in a 20% to 80% improvement in tumor response to drug treatment compared with administration of drug alone in pre-clinical murine models (Bekeredjian et al. 2007; Casey et al. 2010; Heath et al. 2012b; Iwanaga et al. 2007; Park et al. 2012; Sorace et al. 2012). To improve therapeutic effectiveness, novel treatment strategies are needed to overcome the current barriers to drug uptake resulting from tortuous vasculature, limited drug dosages and high tumor interstitial pressure (Jain and Carmeliet 2001, 2012). Pre-clinical *in vivo* cancer research traditionally evaluates treatments through a neoadjuvant animal model by treating primary solid tumors. To date, research into US-stimulated delivery for cancer treatment has focused on improving localized delivery of

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molecules, such as drugs, DNA and viruses (Dalecki 2004; Escoffre et al. 2011; Kinoshita et al. 2006; Lentacker et al. 2008).

In most cancer types, including head and neck cancer (HNC), treatment strategies involve surgery followed by chemotherapy or radiation. Residual disease is common in HNC, and systemic therapy is delivered to further shrink the tumor or treat margins that could not be surgically resected (Vermorken and Specenier 2010). Currently, patients with HNC who undergo surgical resection of a localized tumor have an 80% likelihood of disease recurrence within 2 y (Ridge et al. 2013). To improve overall survival of patients with HNC, it is critical to improve delivery of adjuvant therapy to any residual disease to help improve the therapeutic outcome and reduce cancer recurrence. The devascularized wound bed remaining after surgical removal of a tumor can hinder drug extravasation, further increasing the difficulty of systemic adjuvant treatment.

Ultrasound-stimulated drug delivery has the potential to improve delivery of adjuvant chemotherapy to residual disease and reduce recurrences in HNC. The effectiveness of a therapeutic drug is directly dependent on the amount delivered to the tumor. In the study described here, we investigated the effects of US-stimulated drug delivery on residual disease in a pre-clinical animal model of HNC. The model system detailed provides the basis for advancing pre-clinical surgical models of adjuvant US-stimulated drug delivery in various cancer types with a range of chemotherapeutics. Findings from this study may help advance US-stimulated drug delivery toward clinical translation.

METHODS

Cell preparation

Luciferase-positive HNC cells (squamous cell carcinoma [SCC] 1, provided by Thomas Carey, University of Michigan, Ann Arbor, MI, USA) were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 1% L-glutamate. Cells were passaged at 90% confluency and stored at 37°C and 5% CO_2. Cell counts and viability was determined through hemocytometry and trypan blue dye exclusion.

Animal care and tumor implants

All animal work was reviewed and approved by the Institute of Animal Care and Use Committee, University of Alabama at Birmingham. A 27.5-gauge needle was used to inject 2×10^6 cells/100 μL Dulbecco's modified Eagle medium (without fetal bovine serum) subcutaneously into the right flanks of 5-wk-old female nude athymic mice (Jackson Laboratory, Bar Harbor, ME, USA) (N = 24). Tumor growth was measured biweekly until day 24 using calipers. Given basic tumor diameter measurements along the transverse (d_t) and longitudinal (d_l) dimensions, tumor size was calculated using the equation $\pi \times (d_t/2) \times (d_l/2)$. These caliper measurements reflect tumor size and do not account for necrotic or apoptotic regions.

Surgical resection

Mice were sorted into nine groups: surgical resection (0%, 50% or 100%) + US + drug (N = 3); surgical resection (0%, 50% or 100%) + drug (N = 3); surgical resection (0%, 50% or 100%) alone (N = 2). Average tumor sizes were approximately equal for the different groups (18.7 ± 1.9 mm^2 at day 0). Before surgery, mice underwent baseline imaging and tumor caliper measurements. In mice in the 0% resection group, no tumor was removed, and sham surgery was administered; in mice in the 50% resection group, 50% of the tumor remained; and in mice in the 100% resection group, the entire tumor was removed, as noted by visualization and palpation. Regardless of the group, all mice underwent identical surgical procedures. Briefly, a surgical blade (2976 #15, Feather, Osaka, Japan) was used to open a flap of skin in an 'L' shape, leaving an estimated 1 cm of space around the tumor. The skin flap was sharply dissected from the surface of the tumor, and 0%, 50% or 100% of the tumor was then removed depending on the group. A licensed surgical resident performed each tumor resection. Suturing was completed with 6-O fast-absorbing plain gut suture using a PC-1 conventional cutting 3/8 circle needle (1916, Ethicon, San Angelo, TX, USA). After wound closure, mice were subcutaneously injected near the incision site with a 100-μg cocktail of 1 mg/mL carprofen and 20 μg/mL buprenorphine to relieve residual pain from the surgery. The surgical events are outlined with images in Figure 1. Mice were allowed to heal for 4 d before follow-up experimental studies began.

Cetuximab-IRDye

Cetuximab was labeled with near-infrared IRDye at the UAB Vector Production Facility according to Current Good Manufacturing Practices. This conjugation resulted in 1.8 IRDye molecules per cetuximab molecule. Cetuximab is a monoclonal antibody targeted to the epidermal growth factor receptor (EGFR) and has been approved by the U.S. Food and Drug Administration as a single-agent therapy in HNC.

Ultrasound-stimulated drug delivery

Ultrasound-stimulated drug delivery was applied for 5 min using a 1.0-MHz single-element (19-mm diameter) unfocused immersion transducer (Olympus, Waltham, MA, USA) placed 4 cm from the tumor and the following US exposure parameters: peak negative pressure = 0.9 MPa, pulse repetition period = 15 s and