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A novel pilot animal model for the surgical prevention of lymphedema: the power of optical imaging

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ABSTRACT

Background: Breast cancer–related lymphedema affects more than 400,000 survivors in the United States. In 2009, lymphatic microsurgical preventive healing approach (LYMPHA) was first described as a surgical technique to prevent lymphedema by bypassing divided arm lymphatics into adjacent veins at the time of an axillary lymph node dissection. We describe the first animal model of LYMPHA.

Methods: In Yorkshire pigs, each distal hind limb lymphatic system was cannulated and injected with a different fluorophore (human serum albumin–conjugated indocyanine green or Evans Blue). Fluorescence-assisted resection and exploration imaging system was used to map the respective lymphangiosomes to the groin. Baseline lymphatic clearance of each hind limb lymphangiosome was obtained by measuring the fluorescence of each dye from centrally obtained blood samples. A lymphadenectomy *versus* lymphadenectomy with LYMPHA was then performed. The injections were then repeated to obtain clearance rates that were compared against baseline values.

Results: Human serum albumin–conjugated indocyanine green and Evans Blue allowed for precise lymphatic mapping of each respective hind limb using fluorescence-assisted resection and exploration imaging. Lymphatic clearance from the distal hind limb dropped 68% when comparing baseline clearance *versus* after a groin lymphadenectomy. In comparison, lymphatic clearance dropped only 21% when comparing baseline clearance *versus* a lymphadenectomy with LYMPHA.

Conclusions: We describe the first animal model for LYMPHA, which will enable future studies to further evaluate the efficacy and potential limitations of this technique. Of equal importance, we demonstrate the power of optical imaging to provide real-time lymphatic clearance rates for each hind limb.

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Introduction

Upper extremity lymphedema is one of the greatest survivorship issues amongst breast cancer survivors.[1] Patients who receive axillary node dissection and axillary radiation therapy for breast cancer are at particular risk for the development of lymphedema.[1,2] The psychosocial impact of lymphedema has been described to be as distressing as the initial diagnosis of breast cancer; patients with breast cancer–related lymphedema (BCRL) have a lower quality of life, a higher level of anxiety or depression, a higher likelihood of chronic pain, and greater difficulty functioning socially and sexually compared with breast cancer survivors without lymphedema.[3-10] Lymphedema is characterized by discomfort, functional impairment, and repeated infections.[3] Patients with BCRL incur twice the cost of medical expenses as breast cancer survivors who do not develop lymphedema. Outpatient care, especially mental health services, diagnostic imaging, and visits with moderate or high complexity, account for the difference in the economic impact between these two groups.[11]

The standard-of-care for chronic lymphedema is decongestive therapy with compression garments; however, this is palliative and not curative. Similarly, although functional lymphatic surgery, e.g. lymph node transfer and lymphovenous bypass, improve symptoms and quality of life in patients with chronic lymphedema, these procedures do not offer a definitive cure.[12-14] Therefore, focus has recently turned toward the surgical prevention of lymphedema. In 2009, our Italian colleagues introduced the lymphatic microsurgical preventative healing approach (LYMPHA).[15] For breast cancer patients undergoing an axillary lymph node dissection (ALND), which is the single greatest risk factor for the development of BCRL, divided arm lymphatics are microsurgically bypassed into adjacent veins at the time of the ALND. Four-year follow-up with this technique has demonstrated a lymphedema rate of 4.05% after ALND, and these significant reductions in lymphedema rates have been replicated in the United States.[16-19]

Various preclinical models ranging from rodents to sheep have been developed to study the physiology of lymphedema and its therapeutic intervention.[20] There is, however, an unmet need for the development of an animal model for LYMPHA, as this technique has already revealed early promising clinical results.[16-19] An animal model will allow further study to refine LYMPHA techniques and better understand indications. Moreover, an animal model that allows for real-time evaluation of lymphatic clearance would be optimal. To date, the first and only known animal model that quantifies direct lymphatic flow over time was published in 2009.[21] Using a sheep model and I^{125}, lymphatic flow was measured by injecting the radiotracer into afferent lymphatic channels in the hind limb of the sheep and measuring the radioactivity of blood samples from an internal jugular central line over time. The challenge of obtaining and using nuclear dyes, not only in research but also in clinical practice, greatly limits the utility of this approach.

Our laboratory previously described the use of the fluorescence-assisted resection and exploration (FLARE) imaging system to perform near-infrared (NIR) fluorescence angiography with reliable results.[22-26] We hypothesized that using the same technique with the Federal Drug Administration (FDA)–approved fluorophores, we would be able to obtain real-time lymphatic clearance rates, thereby avoiding the need for nuclear dyes altogether.

The aims of our current study were as follows: (1) to establish the first animal model for the prevention of lymphedema using an immediate lymphovenous bypass after a lymphadenectomy, and (2) to determine the ability of optical imaging agents to quantify lymphatic clearance.

Materials and methods

Preparation of injectable fluorophores

Indocyanine green (ICG, 25-mg vials) and Evans Blue (EB, 25-mg vial) were purchased from Akorn Inc (Lake Forest, IL) and Thermo Fisher Scientific (Waltham, MA), respectively, and used as received. Stock solutions were prepared with sterile water at a 2.5 mg/mL concentration, and transferred to the same volume of 5% human serum albumin (HSA, Sigma-Aldrich) to yield complexes of human serum albumin–conjugated indocyanine green (ICG-HSA) and and Evans Blue (EB-HSA), at the final concentration of 1.25 mg/mL.

Intraoperative NIR imaging system

The basic design and setting of the real-time intraoperative dual-NIR channel imaging system have been described in detail previously.[23,25] In this study, 670-nm excitation light (1 mW/cm^2) and 760-nm excitation light (4 mW/cm^2) were used with white light (400nm-650 nm) at 5500 lux. Color and NIR fluorescence images were acquired simultaneously using AD-130GE camera (JAI Ltd, Yokohama, Japan) installed with custom dual bandpass prism (channel 1: 710/50, channel 2: 780lp), and custom software at rates of up to 15 Hz over a field of view that was manually adjusted by a 3CCD zoom lens (GOYO OPTICAL Inc, Saitama, Japan). In the color-NIR–merged image, 700-nm fluorescence and 800-nm fluorescence were pseudo-colored red and green, respectively. For each experiment, camera exposure time and image normalization was held constant.

Animal preparation

The nonsurvival animal experiments were conducted in compliance with an approved Institutional Animal Care and Use Committee protocol (#030-2016). Yorkshire pigs were quarantined for 48 h as per the Institutional Animal Care and Use Committee guidelines and were given free access to food and water. Animals were fasted 24 h before anesthesia. Female Yorkshire pigs (Parsons EM & Sons Inc, Hadley, MA.) with a mean body weight of 37.2 kg (range, 35kg-39 kg) were induced with 4.4 mg/kg intramuscular Telazol (Fort Dodge Labs, Fort Dodge, Iowa), intubated, and maintained on 2% isoflurane (Baxter International, Deerfield, IL). Electrocardiograph, heart rate, oxygen saturation, and body temperature were monitored during the procedure. Bilateral groins to hind feet were shaved and sterilized with povidone-iodine, and a central venous catheter was inserted into the internal jugular vein. Animals were euthanized with 86 mg/kg intravenous