Intraoperative Near-Infrared Fluorescence Imaging of Thymus in Preclinical Models

Hideyuki Wada, MD, Hoon Hyun, PhD, Homan Kang, PhD, Julien Gravier, PhD, Maged Henary, PhD, Mark W. Bordo, BS, Hak Soo Choi, PhD, and John V. Frangioni, MD, PhD

Division of Plastic and Reconstructive Surgery, Beth Israel Deaconess Medical Center, Boston, Massachusetts; Department of Gastroenterological Surgery II, Hokkaido University Graduate School of Medicine, Sapporo, Japan; Division of Hematology/Oncology, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts; Department of Biomedical Sciences, Chonnam National University Medical School, Gwangju, Republic of Korea; Department of Chemistry, Georgia State University, Atlanta, Georgia; Curadel, LLC, Marlborough, Massachusetts; and Department of Radiology, Beth Israel Deaconess Medical Center, Boston, Massachusetts

Background. **There are currently no thymus-specific contrast agents for biomedical imaging. Thus, finding ectopic thymic tissue during certain operations is extremely difficult. The purpose of the present study was to determine if near-infrared (NIR) fluorescence imaging could provide high sensitivity, real-time identification of thymic tissue during the operation.**

Methods. **After initial in vivo screening of a 315-compound NIR fluorophore library for thymic uptake, methylene blue and five different 700-nm emitting candidate molecules were injected into CD-1 mice for quantitation of the signal-to-background ratio as a function of kinetics and dosing. Results were confirmed in 35-kg Yorkshire pigs. Dual-channel NIR imaging was also performed using a variety of 800-nm emitting NIR fluorophores targeted to various tissues in the mediastinum and neck.**

Results. **The compound Oxazine 170 demonstrated the highest signal-to-background ratio (≥3) for thymic tissue relative to mediastinal fat, heart, lung, muscle, thyroid gland, and parathyroid gland, with peak signal-to-background ratio occurring 4 h after 1 intravenous injection of a human equivalent dose of approximately 7 mg. Simultaneous dual-channel NIR imaging permitted unambiguous identification of the thymus from surrounding tissues, such as endocrine glands and lymph nodes.**

Conclusions. **In mouse and pig, NIR fluorescence imaging using Oxazine 170 permits high sensitivity, real-time identification of thymic tissue for surgical procedures requiring its resection or avoidance. The performance of Oxazine 170 for imaging human thymic tissue is currently not known.**

(Ann Thorac Surg 2016;■:■–■)

The thymus plays a critical role in the development of the immune system, especially T-cell maturation [1–3]. Although thymic tissue is typically located in the anterior mediastinum, ectopic thymic tissue can appear anywhere in the mediastinum and neck, from the level of the thyroid to the diaphragm [4–6]. Despite improved surgical approaches [7], the inability to highlight thymic tissue in the context of surrounding fat and muscle and to locate ectopic thymus results in an unsatisfactory outcome in certain operations, such as those for myasthenia gravis [8–10].

Near-infrared (NIR) fluorescence is a relatively new technology that provides surgeons with high-resolution, high-sensitivity optical imaging in real time. Although NIR light between 700 and 900 nm is invisible to the human eye, special imaging systems can be used to see the light and guide the surgeon. NIR requires the intravenous injection of a fluorescent contrast agent (ie, a chemical or drug) with specificity for a particular target on the surgical field, where it exhibits high uptake in the target of interest.

To date, NIR fluorescence imaging with various contrast agents has been studied in more than 1,000 patients worldwide, in a wide variety of operations. Yet, no studies have been performed for thymus imaging because no thymus-specific contrast agent has been described. In this study, we applied a chemical library screening approach to discover molecules with high uptake in the thymus and conducted preclinical

Dr Frangioni discloses a financial relationship with Curadel, LLC, and Beth Israel Deaconess Medical Center.

The Supplemental Figures can be viewed in the online version of this article [http://dx.doi.org/10.1016/j.athoracsur.2016.09.050] on http://www.annalsthoracicsurgery.org.

Accepted for publication Sept 8, 2016.

Address correspondence to Dr Frangioni, Curadel, LLC, 257 Simarano Dr, Marlborough, MA 01752; email: papers@curadel.com.

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2016.09.050

Abbreviations and Acronyms

Co = cortex
D = distribution coefficient
Fa = fat tissues
FLARE = Fluorescence-Assisted Resection and Exploration
He = heart
LN = lymph nodes
MB = methylene blue
Me = medulla
Ne = nerve
NIR = near-infrared
OX = oxazine
QY = quantum yield
ROI = region of interest
SBR = signal-to-background ratio
TG = thyroid gland
Th = thymus
Tr = trachea

experiments in small and large animals to quantitate contrast agent performance.

Materials and Methods

The animals used in this study were housed in an facility certified by the Association for Assessment and Accreditation of Laboratory Animal Care and were studied under the supervision of the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee in accordance with approved institutional protocols #024-2013 for rodents and #034-2013 for pigs. All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals*.

NIR Fluorescent Contrast Agents

A 315-compound chemical library consisting of polymethine indocyanine, phenoxazine, and phenothiazine derivatives was screened for thymic uptake using CD-1 mice. Methylene blue (MB; 10 mg/mL; 31.3 mmol/L; Taylor Pharmaceuticals, Decatur, IL), was used as a control. Oxazine (OX) 170 and OX750 were purchased from Sigma-Aldrich (St. Louis, MO). OX18, OX27, and OX89 were synthesized in our laboratory. The 800-nm NIR fluorophores for dual imaging were T800-F for thyroid and parathyroid imaging [11] and ZW800-3C for mediastinal lymph nodes [12]. All NIR fluorophores, except MB, were prepared as 10 mmol/L stock solutions in dimethylsulfoxide.

Measurement of Optical Properties

Optical properties were measured in phosphate-buffered saline (pH 7.4) with 5% bovine serum albumin. Quantum yield (QY) of 700 nm and 800 NIR fluorophores was measured using OX725 (Sigma-Aldrich) in ethylene glycol (QY = 19%) and indocyanine green in dimethylsulfoxide (QY = 13%), respectively, under conditions of matched absorbance [13, 14]. In silico calculations of distribution (*D*) coefficient (log*D* at pH 7.4) and three-dimensional minimized structures were performed using Marvin and JChem calculator plug-ins (ChemAxon, Budapest, Hungary).

Animal Models

Male CD-1 mice (n = 57) including three 10- to 12-month-old mice averaging 22 g (Charles River Laboratories, Wilmington, MA) were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine intraperitoneally (Webster Veterinary, Fort Devens, MA) and a median sternotomy was performed to open the mediastinum and thorax. Female Yorkshire pigs (n = 6) averaging 34.8 kg (E.M. Parsons and Sons, Hadley, MA) were induced with 4.4 mg/kg intramuscular Telazol (Fort Dodge Laboratories, Fort Dodge, IA) and intubated. Anesthesia was maintained with 2% isoflurane (Baxter Healthcare, Deerfield, IL). After anesthesia, a 14-gauge central venous catheter was inserted into the external jugular vein, and saline was administered as needed. A median sternotomy was performed for satisfactory intraoperative imaging. Electrocardiogram, heart rate, pulse oximetry, and body temperature were monitored during the experiment.

NIR Fluorescence Imaging System

The dual-NIR channel Fluorescence-Assisted Resection and Exploration (FLARE) (Curadel, LLC, Marlborough, MA) imaging system has been described in detail previously [15, 16]. Color image and 2 independent channels (700 nm and 800 nm) of NIR fluorescence images were acquired simultaneously with custom software at rates up to 15 Hz over a 15-cm diameter field of view. The 700-nm NIR fluorescence and 800-nm fluorescence were pseudo-colored red and lime green, respectively, in merged images.

Intraoperative NIR Imaging of the Thymus in Mice and Pigs

OX170, OX750, OX98, OX170, and OX750 (100 nmol) were injected intravenously into CD-1 mice 4 hours before imaging (n = 3 for each fluorophore). As a control, 100 nmol of MB was injected intravenously 4 hours before imaging (n = 3). For age-related studies, 100 nmol of OX170 was also injected into young (4-week-old) CD-1 mice (n = 3) and adult (10-to 12-month old) CD-1 mice (n = 3). For kinetic studies, 100 nmol of OX170 was quantified over 8 hours (n = 3 mice per each time point). For dose optimization, images were acquired at 4 hours (n = 3 per each dose). Each NIR agent was diluted into 100 μL of saline containing 5% bovine serum albumin before injection.

For large animal studies, 10 μmol of OX170 was injected intravenously into pigs, and thymic tissue in the anterior mediastinum and neck was observed over 8 hours (n = 3). Dual-NIR imaging of the thymus along with surrounding tissues and glands, such as anterior mediastinal lymph nodes, thyroid gland, and parathyroid glands, was performed by injecting 5 μmol of T800-F for thyroid and parathyroid imaging, and 1 μmol of ZW800-3C for lymph node imaging. Dual-NIR images were acquired at 4 hours after the intravenous injection of OX170 and T800-F or