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Non-invasive, dynamic imaging of murine intestinal motility

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Abstract

Background & Aims—After intravenous (i.v.) administration, indocyanine green (ICG) is known to be secreted into bile from the liver via the biliary tracts, enabling fluorescent delineation of the intestine. In addition, ICG is a near-infrared (NIR) excitable fluorophore, capable of providing exogenous contrast for rapid NIR fluorescence imaging. We sought to quantify the intestinal motility using dynamic NIR fluorescence imaging after injection of ICG.

Methods—C57BL6 mice were dynamically imaged immediately before and up to 24 hrs after intravenous (i.v.) and intradermal (i.d.) injection of 50 ul and 10 ul of ICG, respectively. Necropsy was also performed 1 hr post injection and the entire gastrointestinal tract was isolated and exposed for *ex vivo* fluorescence imaging.

Key Results—The secretion of ICG-laden fluorescent bile into the duodenum was observed *in vivo* and confirmed *in situ*. Different patterns of the intestinal motility, such as peristaltic and segmental motions, were dynamically imaged *in vivo*. Our imaging data showed that the frequency of contractions ranged from 27 – 35 cycles/min and the propagation velocity of peristaltic waves ranged from 0.82 ± 0.5 to 2.04 ± 1.12 cm/s.

Conclusions and Inferences—Dynamic NIR fluorescence imaging with injection of ICG can provide a method for diagnostic motility testing for intestinal motility disorders or dysfunction and for potential evaluation of therapeutic agents.

Keywords

Near-infrared fluorescence imaging; indocyanine green; intestinal motility; bile secretion

Introduction

The gastrointestinal (GI) tract has various controlled motions to appropriately mix and propel its contents as an essential part of digestion and absorption. Different patterns of intestinal motions, such as peristaltic and segmental, have been described and are shown to be the result of interplay among smooth muscle cells, interstitial cells of Cajal (ICCs), and enteric neural circuits [1]. Normal intestinal motility can be influenced by pathological conditions including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and chronic intestinal pseudo-obstruction [2, 3].

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Author contributions

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Intestinal motility function has been extensively assessed on the isolated segments of rat, guinea-pig, and mouse intestines by measuring electrical signals with or without video imaging for construction of spatio-temporal maps of changes in diameter [4–7]. Several groups have reported non-invasive dynamic x-ray imaging of intestinal motility in conscious mice after oral gavage of barium or iodine solution [8–10]. Besides the difficulties of repeated oral contrast agent administration, artifactual intestinal motions may be observed in these studies due to the distension of the intestinal walls by the significant quantities of contrast. While several murine animal models have been developed to study gut motility and how it may be impacted by potential pharmacological agents, there remains a need to develop better non-invasive and long-term assessment tools of intestinal motility. Recently, we developed a dynamic near-infrared (NIR) fluorescence imaging technique with injection of indocyanine green (ICG), which we have utilized to assess lymphatic function in mice [11, 12] and in humans [13–16]. ICG is a NIR fluorophore that is excited at 780 nm and emits fluorescence at 830 nm, which is advantageous for non-invasive tissue imaging due to low tissue absorption and scattering and lack of background fluorescence. ICG is approved in humans for diagnostic purposes not only on the basis of its fluorescent properties, but on the basis of its dark green color for vascular imaging and clearance studies following i.v. administration, due to its association with plasma proteins [17–21].

Since bile enters the duodenum via the biliary tracts, ICG-laden fluorescent bile can delineate the intestine, enabling non-invasive imaging of intestinal motility. Herein we show non-invasive, dynamic NIR fluorescence imaging of contractile activity in the murine intestine after i.v. or i.d. injection of ICG. This imaging technique can be used to longitudinally and quantitatively assess motility in animal models of intestinal disorders and to potentially provide diagnostics and information in response to pharmacological intervention.

Materials and methods

Animals

Female C57BL6 mice (4–6 wk old; Charles River, Wilmington, MA) were housed in a pathogen-free mouse facility accredited by the American Association for Laboratory Animal Care and maintained in standard cages with free access to water and pellets prior to imaging. Animal experiments were approved by and performed in accordance with The University of Texas Health Science Center Institutional Animal Care and Usage Committee and in accordance with NIH guidelines.

In vivo dynamic NIR imaging

Mice were anesthetized with isoflurane and maintained at 37 °C on a warming pad. A volume of 10 µl or 50 µl of 645 µM of ICG (Akorn, Inc. Buffalo Grove, IL) dissolved in mixture of distilled water and 0.9 % Sodium Chloride in a volume ratio of 1:9 was injected intradermally and intravenously, respectively.

For i.v. injection of ICG, a catheter was placed in the tail vein of anesthetized mice to facilitate the subsequent delivery of the fluorescent dye. Dynamic fluorescence images were acquired for a period of approximately 6 min immediately before and up to 24 hrs after i.d. or i.v. injection using a custom-built NIR fluorescence imaging system described elsewhere (Supplemental Figure 1) [11, 12]. We also measured abdominal skin temperature using an infrared thermometer and observed no temperature changes during dynamic fluorescence imaging. Since non-invasive imaging of the intestines may not distinguish the GI lumen from blood and lymphatic vessels, necropsy of the mouse with exposure of the abdomen was

performed at 1hr post injection (p.i.) and the GI tract was removed and laid out on a black paper for intravital imaging.

Data analysis

The data were analyzed with a custom Matlab (The MathWorks, Inc., Natick, MA) program and ImageJ (National Institutes of Health, Bethesda, MD). Fixed regions of interest (ROIs) of equivalent areas were selected along the length of the fluorescent intestine. The three-dimensional plot of fluorescent intensity as a function of time and length of the intestine was generated and used as a spatio-temporal map to assess the propagation velocity and the frequency of the propagation along the intestine. Respiratory movements were not corrected for in the construction of the 3-D spatio-temporal maps. The packet of fluorescent contents is represented by the peak in fluorescence intensity which propagates along the intestine with time. Two ROIs were identified, from which the distance and time information was extracted to measure the propagation velocity. Although our non-invasive imaging of intestinal motions needs to be confirmed by supplementary measurements such as slow wave frequency and slow wave propagation velocity, which need to use invasive methods, peristalsis was defined as aboral propagation of contraction waves along the intestine in this study.

Results

After i.v. injection of ICG, rapid distribution of the ICG throughout the mouse was observed within seconds as shown in Supplemental movie #1. Figure 1A shows a fluorescent image showing blood vessels in the skin and gut at 4 sec p.i. Figure 1B represents overlay of white light and fluorescent images of the mouse with exposure of the abdomen at 1hr p.i., showing fluorescent signal in the biliary system (also shown in magnified fluorescent images in Figure 1C) and distribution of fluorescence in the intestines. Strong and non-uniformly distributed fluorescence arising from its contents, but not from the intestinal tissues was detected throughout the intestine with weak and no fluorescence in the cecum and colon, respectively, at 1hr p.i.. Fluorescent signal arising from the stomach was absent at all times.

Figure 2A represents selected frames taken of mice after i.v. or i.d. injection of ICG, showing intestinal transit of ICG at different times after administration. Supplemental movie #2 demonstrates the contractile function of the small intestine 30 min after i.d. injection, showing secretion of ICG-laden bile into the duodenum. Vigorous contractility of the fluorescent small intestine was observed 1hr and 3hr after i.d. injection as shown in supplemental movie #3 and #4. Non-invasive fluorescence imaging of the intestines may not provide accurate information about the diameter of the intestines, due to absorption and scattering of light by tissues. However, intestinal contractility can be determined. A series of ROIs were selected on the static images similar to the analysis conducted in dynamic x-ray fluoroscopy [9, 10]. Figure 2B depicts an example of the fluorescent image, where 76 ROIs were selected on the fluorescent small intestine in the same animal shown in Supplemental movie #3. Figure 2C shows the spatial-temporal map of fluorescent intensity denoted in color with x-axis detailing position along the intestine and y-axis the time of imaging. The maximum intensity signifies “waves” of ICG transit, demonstrating the aboral propagation of the contractile wave (arrow) along the small intestine at the propagation velocity of 2.04 ± 1.15 cm/s and 27 cycles/min in this mouse. The frequency of peristalsis and propagation velocity in the duodenum as shown in Supplemental movie #1 was 35 cycles/min and 0.82 ± 0.5 cm/s, respectively. Figure 2D shows another example of the spatial-temporal map of fluorescent intensity along the small intestine, demonstrating segmental contractions as shown in Supplemental movie #5.

Discussion

In this report, we demonstrated for the first time, non-invasive NIR fluorescence imaging with sufficient temporal resolution and sensitivity to assess dynamic motion of the murine intestine, due to delineation of the intestinal wall resulting from the secretion of bile containing ICG from the liver after i.v. or i.d. injection of ICG. Our NIR fluorescence imaging system can be easily combined with a traditional, invasive video imaging method, similar to that reported by De Backer et al. [22]. In their studies, however, intestinal motions could not be non-invasively imaged with oral administration of fluorescein- dextran, due to limited tissue penetration depth of the visible light exciting fluorescein and tissue autofluorescence within the range of emission wavelength. Therefore, our NIR imaging system combined with a video imaging technique may provide a better method to evaluate murine intestinal motility. Although we did not gavage ICG in our study, oral gavage of small amount of ICG solution may be another method especially for imaging gastric emptying and dynamic transit of ICG along the GI tract [23], eliminating the background fluorescence associated with i.d. or i.v. injections. However, the low pH of the stomach may preclude *in vivo* fluorescence imaging [24]. Similar to ICG, Tc^{99m} labeled iminodiacetic acid derivatives, such as mebrofenin, is also secreted with the bile and has been used to image intestinal motions in patients after i.v. injection using dynamic scintigraphy (i.e., 10 sec time interval between images); however, different patterns of intestinal motions and quantification of the motions cannot be elucidated, due mainly to relatively poor temporal resolution [25, 26].

Previous *in vivo* dynamic fluoroscopy after oral administration of barium or iodine solution to intact mice showed the frequency of small intestinal contractions of 44.1 ± 1.3 to 47 ± 1 cycles/min and the propagation velocity of 1.09 ± 0.1 cm/s to 1.6 ± 0.1 cm/s [8–10]. Our fluorescence imaging data are in general agreement showing the contraction frequency and the propagation velocity are 27 to 35 cycles/min and 0.82 ± 0.5 to 2.04 ± 1.15 cm/s in the small intestine. Although the frequency of peristaltic contraction measured in this study may be lower than that reported by others, care should be taken when comparing results obtained under different measurement, imaging conditions, and anesthesia. Herein the use of isoflurane could have an impact upon peristaltic contractions [27].

While non-invasive ICG fluorescence imaging has been reported in humans [28], it is uncertain whether excitation light penetration is sufficient for human abdominal imaging. The current limitation may exist in the instrumentation rather than medical physics. For example, while nuclear imaging focuses upon the collection of single photon events following decay of radionuclides, fluorescent dye molecules can be repeatedly excited and release as many as 100,000,000 photons/sec. The realization of imaging sensitivity may lie in improving imaging instrument design [29]. Currently, we have estimated detection of ICG at 4–5 centimeters of tissue depth in investigational human studies [13]. Therefore, NIR fluorescence imaging holds the promise of a highly sensitive, non-ionizing imaging modality capable of real-time *in vivo* imaging with small volumes and amounts of contrast agents as compared to dynamic fluoroscopy and scintigraphy.

In conclusion, non-invasive NIR fluorescence imaging with injection of ICG can be used to image intestinal motility and can provide a method for diagnostic motility testing for intestinal motility disorders or dysfunction, and development and evaluation of therapeutic agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) Fluorescent image frame showing blood vessels 4 secs after i.v. injection of ICG.

Arrow: superficial blood vessels. Broken arrow: blood vessels in the GI. (B) Overlay of white light and fluorescent images showing the biliary system and the intestine in a mouse with exposure of the abdomen 1 hr after i.v. injection. (C) Magnified fluorescent image of the rectangle in Figure 1B showing the biliary system. Scale: 1mm. CBD: common bile duct. CD: cystic duct. CE: cecum. DU: duodenum. GA: gallbladder. HD: hepatic duct. IT: intestinal tissues. LI: liver.

Figure 2.

(A) Overlay of white light and fluorescent images 30 min, 1 hr, 3 hr, 5 hr, and 24 hr after i.d. and i.v. injection of ICG, showing GI transit of ICG. I.v. injection of the small amount of ICG did not induce mass transport of ICG-laden bile to the intestines. However, i.d. and i.v. administrations resulted in different clearance rates as expected. When ICG was injected intravenously, the fluorescent signal in the liver was greatly reduced at 1 hr p.i., whereas fluorescence was still high in the liver and gallbladder after i.d. administration, due to continuous lymph formation after i.d. injection, clearance back to blood circulation via subclavian vein, and finally excretion by the liver into bile. (B) Fluorescent image showing the placement of 76 ROIs on the fluorescent small intestine. (C, D) Fluorescent intensity map as a function of time and the length of the intestine, demonstrating aborally propagating peristaltic waves (C; solid arrows) and segmental contractions (D).