Novel Lymphography Using Indocyanine Green Dye for Near-Infrared Fluorescence Labeling

Fusa Ogata, MD,* Ryuichi Azuma, MD,† Makoto Kikuchi, PhD,* Isao Koshima, MD,‡ and Yuji Morimoto, MD, PhD§

Abstract: Lymphedema is known to be caused by many pathologic conditions; however, its diagnostic and therapeutic strategies remain to be unestablished. In this study, we investigated the usefulness of a novel lymphographic method based on fluorometric sensing using indocyanine green (ICG) dye for imaging lymphatic vessels using rat models. The real-time imaging system enabled visualization of superficial lymphatic vessels with a diameter of 0.1 mm in 33 frames/second. In addition, morphologic changes in lymphatic vessels in a radiation-induced lymphedema model were detected even at the latent stage. These results suggest that this imaging technique is acceptable as an evaluation method for the lymphatic system.

Key Words: lymphedema, indocyanine green, lymphography, near-infrared fluorescence, rats

(Ann Plast Surg 2007;58: 652–655)

Anatomic and/or functional obstruction in lymphatic circulation results in slow progressive accumulation of protein-rich interstitial fluid, a condition known as lymphedema. This lymphatic blockage is induced by several pathologic conditions such as surgery, radiation, trauma, and infectious diseases.

In cases of lymphedema, morphologic changes in lymphatic vessels are frequently observed before the emergence of clinical manifestations. These changes include abnormal dilation, curvature of the lymphatic tract, and abnormal retention of a contrast agent for imaging.1 Thus, if morphologic changes can be detected in the latent stage of the disease, it should be possible to initiate treatment to prevent the onset of clinical manifestations. Early initiation of treatment is recommended since several studies have shown a good correlation between amelioration of lymphedema and initiation time of treatment.2,3

Conventional methods such as lymphangiography and lymphoscintigraphy have been used for detection of morphologic abnormalities in lymphatic vessels. Lymphangiography enables visualization of the lymphatic system with high resolution, but this invasive technique is no longer in use because it has frequently induced serious complications such as damage to the lymphatic tunica interna and pulmonary oil embolism. Lymphoscintigraphy has recently become the imaging modality of choice for evaluating lymphatic function, but the spatial resolution is not necessarily satisfactory; it does not allow the discrimination of lymphatic vessels but can at best enable visualization of abnormal lymph-liquid pooling, indicating the difficulty in detecting a stasis point in a lymphatic vessel. Therefore, a less invasive imaging method with high spatial resolution is needed.

Here we propose a novel lymphographic method based on fluorometric sensing using indocyanine green (ICG) dye. Recently, an ICG fluorescence high-sensitivity near-infrared video camera system (PDE; Hamamatsu Photonics, Hamamatsu, Japan), in which the optical filtering system is optimized, has been developed. Since fluorescence maximum of ICG is within the near-infrared spectral range (800 nm) and human tissue is relatively transparent for near-infrared light, this system enables noninvasive detection of the fluorescence in deep dermal layers. In fact, the PDE system has been shown to enable detection of deeply located (2 cm) sentinel lymph nodes from the body surface of patients with breast cancer.

The purpose of this study was to develop a real-time optimal imaging method to detect lymphatic vessels using the ICG fluorescence-sensitive camera system. Using this system, we succeeded in visualizing superficial lymphatic vessels in rats, with high spatial resolution. In addition, using this system, we detected acute lymphatic damage even in a latent stage of radiation-induced lymphostasis.

MATERIALS AND METHODS

ICG Fluorescence High-Sensitivity Near-Infrared Video Camera System

Lymphatic vessels were visualized using the PDE system, which is equipped with a CCD camera as a fluorescence detector, with a low-cut filter below 820 nm and 760-nm LEDs as a light source for emission of ICG. The fluorescence images were digitized using a standard personal computer with a time resolution of 33 ms. The system has been shown...
to enable detection of sentinel lymph nodes located ∼2 cm from the body surface.5

**Lymphedema Rat Model**

We made an acute obstructive edema model by modifying previously described methods.6,7 The left groins of rats under anesthesia induced by intraperitoneal injection of pentobarbital (50 mg/kg) were radiated with 30–50 Gy as a single dose. The ionized radiation was emitted from an x-ray machine (MBR-1505 R2; Hitachi, Japan) at a dose rate of 3.15 Gy min⁻¹ (150 kVp, 5 mA). The unexposed right extremity in the same animal served as a control. One week later, the following operative procedures were carried out for facilitating lymphedema. First, 0.1 mL of 100-mg/mL Evans blue (EB) dye was injected into the subcutaneous tissue of the left paw for identification of lymphatic vessels. The left inguinal skin was then obliquely incised, and lymphatic vessels entwined around the left femoral neurovascular bundle were easily confirmed. The stained lymphatic vessels were carefully tied at 3–8 points (average, 5 points per lymphatic vessel) with 8-0 nylon suture, and the inguinal and popliteal lymph nodes were completely resected. The wounds were closed by suturing the stumps into muscles. Lymphedema formation was quantitatively evaluated by measurement of the circumferential length of the hind limb at the level of 15 mm proximal to the heel.

**Imaging for Normal Rat and Lymphedema Rat**

In the rats under anesthesia induced by intraperitoneal injection of pentobarbital (50 mg/kg), 0.1 mL of a 2.5-mg/mL solution of ICG was injected subcutaneously into the first web space of the foot. The foot was then observed from the upper surface using the PDE camera system.

The animal study protocols were approved by the Ethics Committee for Laboratory Animals of the National Defense Medical College, Tokorozawa, Japan.

**RESULTS**

**Fluorescence Images of Normal Rats**

Immediately after ICG injection to the first web space of the foot, a bright spidery spot was seen on the dorsum of the foot. A few seconds after tapping the dorsum of the foot, lymphatic flow was visualized as ICG fluorescence (Fig. 1B), reaching the region of the popliteal lymph nodes. For confirmation of lymphatic vessels, 0.1 mL of a 100-mg/mL solution of EB was injected into the first web space 15 minutes after the injection of ICG. On the fascia, EB-stained lymphatic vessels were seen where expected (Fig. 2B). As a result, a lymphatic vessel with a diameter of more than 0.1 mm was detected using the near-infrared camera system. The sensitivity seems to be almost 100% since we identified ICG fluorescence from lymphatic vessels in all of the examined rats (n = 20) by confirming EB staining. In addition, specificity is presumed to be 100% because if ICG is injected into arteries or veins, the dye is immediately washed away within a minute, causing immediate blurring and disappearance of the fluorescence; however, ICG remains inside lymphatic vessels for at least over 10 minutes, allowing visualization of fluorescence for 10 or more minutes.

**Fluorescence Images of Lymphedema Rats**

In the lymphedema model, apparent swelling of the radiated hind limb was not visually confirmed until 4–5 days after lymph node resection (11–12 days after ionized radiation): the circumferential length of the treated hind limb was less than 110% of the pretreated size. In the latent stage of lymphedema, however, the ICG fluorescence camera system revealed significant changes in lymphatic vessels even at 3 days after lymph node resection. As shown in Figure 3B, fluorescent lymphography revealed disappearance of the ma-
major lymphatic trunks that had been confirmed at the preradiation phase. Instead, moniliform lymphatic microvessels, parts of which were tortuous, were newly confirmed. Swelling of the radiated hind limb became visually apparent 7 or more days after lymph node resection in this model. In the apparent swelling phase, vessel structure was no longer seen in the fluorescent lymphograph, replaced by a bright and punctulate fluorescence pattern with a foggy background (Fig. 3C).

FIGURE 2. Lymphatic vessels corresponding to lymphatic flow in a normal rat. A, Enlarged view within the box in Figure 1B. B, Exposed superficial lymphatic vessels filled with EB. *2-0 Nylon suture (0.300–0.399 mm in diameter). **Small saphenous vein.

FIGURE 3. ICG image in lymphedema rat model. A, In preradiated period. B, At 3 days after lymph node resection. C, At 7 days after lymph node resection. Apparent damage to lymphatic vessels was confirmed at 3 days after lymph node resection (B), but there was no apparent swelling of the hind limb at that time. At 7 days after lymph node resection, swelling of the hind limb became evident. Circumferential lengths of the lower thigh (1.5 cm proximal to the heel) were 2.9 cm, 2.7 cm, and 4.2 cm in the preradiated period, 3 days after lymph node resection, and 7 days after lymph node resection, respectively. These changes were seen in 3 separate rats.

The sensitivity of the system used in the present study was considered to be very high. Since there is no report on ICG fluorescence-based lymphography, further studies are required for performance evaluation; however, a related study has shown that the detection rate of lymphatic channels in sentinel lymph node biopsy is 100%. The specificity of the system also seems to be high. As mentioned above in the Results, by sequential monitoring of fluorescence images for 10–20 minutes, lymphatic vessels can be discriminated from arteries and veins.

Using ICG-enhanced near-infrared lymphography, we identified lymphatic tracts from outside the body without skin incision. This less invasive imaging system can provide real-time vision at 30 frames/second and enables visualization of lymphatic vessels with a diameter of 0.1 mm. The fluorescence lymphography enabled visualization of not only normal lymphatic vessels but also damaged ones. Furthermore, this imaging system enabled detection of lymphostasis even in the latent stage.

The fluorescence-based lymphography is almost noninvasive and can be performed easily. In addition, this method can be repeatedly used in the same patient. The PDE system used in the present study is small and portable, thus allowing convenient use anywhere.

Since ICG needs to be injected for the examination, the occurrence of complications should be anticipated; however, intravenous injection of ICG is routinely used for clinical examination of hepatic function, and the complication rates
are very low (0.17%), most of (0.14%) of the complications, including nausea (0.08%), fever (0.02%), and angialgia (0.02%), not being severe. Although shock-related complications (0.02%) have also been reported, they are not fatal.8

The results of this study suggest that this imaging technique is acceptable as an evaluation method for the lymphatic system. This sensitive imaging method enables detection of lymphedema at the latent stage and therefore appears promising for use in both a clinical setting and in experimental models.

ACKNOWLEDGMENTS

The authors appreciate the advice and technical comments about ionized radiation from Dr. Manabu Kinoshita, National Defense Medical College Research Institute.

REFERENCES
