Visualization of subsurface blood vessels by color Doppler optical coherence tomography in rats: before and after hemostatic therapy

Richard C. K. Wong, MBBS, Siavash Yazdanfar, MS, Joseph A. Izatt, PhD, Manish D. Kulkarni, PhD, Jennifer K. Barton, PhD, Ashley J. Welch, PhD, Joseph Willis, MD, Michael V. Sivak, Jr., MD

Background: The ability to visualize subsurface blood vessels and measure flow may be useful in certain experimental and clinical settings.

Methods: Color Doppler optical coherence tomography was used to visualize and measure blood flow in subsurface vessels in vivo in a rat skin flap model. Local “hemostatic” interventions (epinephrine or sclerosant injection, heat probe, and laser) were then applied and imaging was repeated. The skin flap was evaluated histologically.

Results: Subsurface blood vessels were easily visualized in cross-section, and vessel diameter and bidirectional blood flow velocity were readily measured. Color Doppler optical coherence tomography demonstrated that flow was significantly reduced after epinephrine injection and became undetectable after the other interventions. This correlated with pathologic evidence of vessel damage in all interventions, except for epinephrine injection. Although vessel response was as predicted to most interventions, the response to epinephrine was only temporary, and limited application of heat alone from the heat probe halted flow without visually apparent surface injury.

Conclusions: Color Doppler optical coherence tomography provides high-resolution, cross-sectional flow imaging in subsurface blood vessels. Color Doppler optical coherence tomography is potentially a better technique for the study of existing and new hemostatic intervention in the laboratory. Potential future clinical applications include monitoring of the response to hemostatic modalities.
Endoscopic therapy for the primary control of acute peptic ulcer bleeding is highly effective with rates approaching 100%. Nevertheless, recurrent bleeding remains a significant problem because it has been identified as a major independent predictor of mortality. Persistent or recurrent bleeding has been estimated to occur in 21% of patients after one session of endoscopic therapy for the actively bleeding ulcer or nonbleeding visible vessel. In part, this may explain why the overall mortality rate from bleeding peptic ulcers has remained largely unchanged around 6% to 7% for the past 30 years.

By using the endoscopic Doppler US probe in patients presenting with acute peptic ulcer hemorrhage, it has recently been demonstrated that a persistently positive Doppler signal after endoscopic therapy indicates a very high risk of recurrent bleeding. However, Doppler US is unable to actually visualize subsurface blood vessels. The ability to visualize ulcer-associated submucosal blood vessels in patients presenting with acutely bleeding ulcers, may allow for precise and potentially more effective and long-lasting endoscopic therapy. At present, there is no proven practical method for imaging ulcer-associated submucosal blood vessels in the human GI tract. Some investigators have used near-infrared imaging systems to visualize subsurface blood vessels. Color Doppler optical coherence tomography (CDOCT) has been used to demonstrate subsurface blood vessels in the human retina, human and animal skin, and in the rat cerebral cortex. CDOCT (also called optical Doppler tomography) is a novel technique for simultaneous imaging of cross-sectional tissue structure and for the determination of the presence or absence of flow in subsurface blood vessels. In addition to measuring vessel diameter, CDOCT can identify the direction of flow and estimate flow velocity. CDOCT has been used in experimental studies (in organs other than the GI tract) to evaluate the effect on blood vessels of various interventions such as pulsed dye laser irradiation, photodynamic therapy, and administration of nitroglycerin.

A preliminary in vivo study was conducted in animals to determine whether CDOCT could be used to monitor the effects on blood flow of certain “hemostatic” interventions used in GI endoscopy. Interventions were chosen that are commonly used in the endoscopic treatment of acutely bleeding ulcers such as epinephrine or sclerosant injection, heat probe thermal coagulation, and laser photoagulation. Our aim was to determine the potential for application of CDOCT to clinical medicine, in particular the treatment of GI bleeding. In addition, our aim was to ascertain whether CDOCT could be used to study basic mechanisms by which endoscopic hemostasis is achieved, thereby potentially improving their effectiveness.

**MATERIALS AND METHODS**

**High-resolution optical flow imaging**

High-resolution optical flow imaging is based on the principles of optical coherence tomography (OCT), a technique that provides high-resolution, cross-sectional tissue imaging. In OCT, a broadband light source illuminates a fiber optic Michelson interferometer, as illustrated in Figure 1. Half of the light is directed into a reference arm containing a mirror and the other half into a sample arm containing the biologic tissue. Back-scattered light from each of the arms interferes constructively or destructively at the detector only when the optical path lengths of the two arms are matched within the source coherence length. The axial resolution is thus limited by the coherence length, which is inversely related to the bandwidth of the source.

Our OCT system uses a 1.2 mW superluminescent diode with a 37 nm bandwidth (full width at half-maximum) centered at 1.27 µm wavelength, which corresponds to a source coherence length of 19.2 µm. Depth localization is achieved by linearly scanning the reference arm length while recording the interference of light reflected from the sample and reference arms. This creates interference patterns at the detector, which correspond to internal reflections in the sample as a function of depth, similar to A-mode US. By demodulating the detector current at the constant Doppler frequency induced by the motion of the reference arm mirror, the tissue reflectivity is measured as a function of depth. Lateral scanning of the sample beam across the tissue while recording the detector current results in two-dimensional, cross-sectional images of tissue microstructure. The lateral resolution is determined by the spot size of the beam incident on the tissue, or 14 µm. This method of imaging has exceptional sensitivity (~110 dB), being able to detect tissue backscatter signals on the order of tens of femtowatts (1 fW = 10^{-15} W).
CDOCT is an extension of OCT, in which blood flow velocities are quantified by estimating Doppler shifts in the back-scattered light of the sample arm. It is based on the principle that Doppler shifts in reflections from a biologic sample will, depending on the direction of the flow, add to or subtract from the constant Doppler frequency associated with the moving reference arm. The Doppler shift in the sample arm, $f_s$, is related to the flow velocity, $V_s$, by:

$$f_s = \frac{2n_r V_s \cos \theta}{\lambda_0}$$

where $n_r$ is the mean tissue index of refraction, $\lambda_0$ is the center wavelength of the source, and $\theta$ is the angle between the incident light and the direction of motion of the scatterers. Hence, by measuring the mean frequency as a function of depth, one can obtain velocity estimates within the tissue.\textsuperscript{12,13,15}

**Creation of the dorsal skin flap model**

This study was approved by our Institutional Review Board for Animal Investigation. Three Sprague-Dawley rats weighing approximately 250 g each were implanted with dorsal skin flap windows.\textsuperscript{20} The window was supported by two aluminum plates, which were bolted together and attached surgically to a double-thickness dorsal skin flap. One plate had a housing to accept a 1-cm diameter glass or plastic window, which was affixed with an aluminum retaining ring. The implantation procedure was accomplished with the animals under deep anesthesia by using a 3:4 ratio mixture of xylazine [20 mg/mL] and ketamine hydrochloride [100 mg/mL] (0.1 mL per 100 g, intramuscular injection). Repeat injections were administered, as required, to keep the rats under deep anesthesia. Briefly, the implantation procedure entailed removing the hairs on the back of the rat, grasping and lifting the dorsal mid-line skin (creating a double-thickness skin flap), which was then sutured to a temporary C-clamp. Bolt holes were then punched through the double-thickness skin flap and a 1-cm circle of skin was cut from one side. This allowed for the exposure of the subdermal tissue of the opposing single-thickness skin flap. The aluminum plates were bolted and sutured in place, the temporary C-clamp was removed, and a glass or plastic window was set in place. All the CDOCT images in this study were obtained by scanning from the window (or subdermal) side of the preparation. During CDOCT imaging, the anesthetized rats were placed in a secure plastic apparatus designed to firmly hold the window in place at a known angle to the optical axis of the CDOCT probe (thus minimizing movement artifacts during scanning). Multiple observations can be made in each rat from several dorsal skin flap windows.

**Analysis of CDOCT images**

The images were acquired with a reference arm scanning at 32.5 mm/s and were composed of 512 pixels in depth and 200 pixels across. This resulted in an acquisition time of 10 to 20 seconds per image. The axial dimensions represented optical depth (physical distance or depth was obtained by dividing optical depth by the bulk index of refraction of the tissue). Structural information attained by conventional OCT was encoded logarithmically in gray scale, in which white indicated high reflectivity and black indicated low reflectivity. For CDOCT velocity information, the direction and magnitude of blood flow were encoded by color (red or blue) and color intensity, respectively. Before combining the color scales into a single image, the velocity data were threshold-adjusted to remove noise. For each specific experimental intervention, the noise threshold was kept constant in the preintervention and postintervention images.

**Experimental hemostatic interventions**

After the creation of the dorsal skin flap window, photodocumentation was obtained of the window using a surgical microscope. The anesthetized, prepared rats were individually placed in a secure plastic holder with the dorsal skin flap exposed. The vessel(s) of interest were identified and CDOCT scanning performed with the light beam incidence at approximately $\theta = 60$ degrees to the longitudinal axis of the vessel(s). The various local hemostatic interventions were then performed with the rats secured in this apparatus. CDOCT scanning was repeated at several intervals after each therapy. The spatial relationship of the CDOCT scanning light beam and the dorsal skin flap was kept constant for each individual animal and during the experimental interventions. One animal was used for each method of intervention. The animals were kept heavily anesthetized for the duration of the entire experiment, and then they were sacrificed without awakening at the end of each individual experiment. Photodocumentation of the window was again obtained at the end of each experiment. The scanning path of the CDOCT light beam across the index vessel(s) was carefully marked at the end of each experiment with surgical sutures. The dorsal skin flap window was then cut out and placed in 10% formalin. After fixation, careful sectioning was then performed across the index vessel(s) at the site marked by the surgical sutures. These microscopic tissue sections were then processed and stained (hematoxylin and eosin) with standard histopathologic techniques. Photodocumentation was obtained of the stained sections by using a surgical microscope.

**Epinephrine injection**

By using a 1.0-mL tuberculin-type syringe and needle, 0.1 mL of 1:100,000 dilution of epinephrine was injected (from the skin side) into the subsurface tissue adjacent to the index vessels. CDOCT scanning was performed before and after injection.

**Sclerosant injection**

By using a 1.0-mL tuberculin-type syringe and needle, 0.05 mL of sclerosing solution (sodium tetradecyl sulfate, alcohol, and saline mixture) was injected from the skin side into the subsurface tissue adjacent to the index vessels. CDOCT scanning was performed before and after injection.

**Thermal coagulation**

Thermal contact coagulation was performed with a commercially available heat probe unit with a 7F heat probe...
(Olympus Optical Co., Ltd., Tokyo, Japan). This probe was lightly applied to the index vessels (from the window side), with settings of 10 J × 2 pulses, followed by 20 J × 3 pulses. CDOCT scanning was performed 3 times: before therapy; after 10 J × 2 pulses; and after 20 J × 3 pulses.

**Laser application**

A single 600 µm optical fiber was used to deliver laser pulses (Nd:YAG at 1064 nm) to the index vessel from the window side. The fiber was directed at the index vessel and the distal end was held by hand within 5 mm from the tissue surface for an approximate spot size on the tissue of 1 to 2 mm. The laser was set at 5 W and 2 timed pulses were delivered, of 2- and 5-second duration each. CDOCT scanning was performed 3 times: before therapy, after 2-second therapy, and after an additional 5 seconds of therapy.

**RESULTS**

**CDOCT imaging of subsurface blood vessels**

CDOCT visualized subsurface blood vessels in cross-section as rounded structures with color Doppler signal indicating direction of flow (Fig. 2A). The vessels ranged in cross-sectional diameter from 0.28 to 0.59 mm. Bidirectional peak blood flow velocities ranged from –1.9 to +1.9 mm/s. Minor velocity noise (or artifact) was seen in some of the CDOCT images from the epinephrine and the heat probe treatment groups. This was caused by a mechanical disturbance in the movement of the scanning reference minor, which was corrected before beginning the laser treatment experiment.

**Epinephrine injection**

The pretreatment CDOCT image clearly demonstrated 2 blood vessels with bidirectional blood flow (Fig. 2A). CDOCT images taken during the first hour after epinephrine injection did not show evidence of detectable flow (result not shown). Subsequent images taken at 3.0 and 4.5 hours after treatment did demonstrate the resumption of minimal blood flow in both vessels (Fig. 2B). The peak flow velocity was significantly reduced from 1.28 mm/s (before treatment) to 0.63 mm/s (after treatment) in one of these vessels. The corresponding visual image of the posttreatment subdermal vasculature appeared unchanged (result not shown). Standard histologic section taken directly across the CDOCT scan path (after treatment at 4.5 hours) demonstrated 2 normal-appearing blood vessels without evidence of intraluminal thrombosis or surrounding inflammatory response (Fig. 2C).

**Sclerosant injection**

The pretreatment CDOCT image demonstrated 2 vessels with bidirectional blood flow. After the injection of sclerosant solution, the surface tissue appeared erythematous, and repeat CDOCT imaging demonstrated no blood flow. The corresponding histologic section showed 2 vessels in a region with extensive necrosis (result not shown).

**Heat probe application**

The visual and CDOCT images of the subdermal vasculature demonstrated 2 blood vessels with bidi-
rectional flow (CDOCT image is shown in Fig. 3A). After the application of the heat probe set at 20 J (10 J × 2 pulses), there was only minor tissue damage; however, blood flow was undetectable (Fig. 3B). The visual appearance of the surface appeared unchanged (result not shown). Therefore, an additional 60 J (20 J × 3 pulses) was applied with the heat probe. This resulted in the visual appearance of a definite surface crater, undetectable blood flow, and OCT findings consistent with subsurface tissue vacuolization (Fig. 3C). The corresponding histologic section demonstrated 2 blood vessels with surrounding extensive cautery artifact (Fig. 3D).

Laser application

The pretreatment visual and CDOCT images demonstrated one single blood vessel (CDOCT image is shown in Fig. 4A). After the initial laser application of 5 W for 2 seconds, there was still detectable blood flow (result not shown). However, after the additional laser application of 5 W for 5 seconds, blood flow was undetectable by CDOCT imaging (Fig. 4B). Visually, the damage to the blood vessel was focal and localized to one short segment; the surrounding tissue surface appeared relatively intact (Fig. 4C). The corresponding histologic section demonstrated a portion of vessel with extensive coagulative necrosis at the site of laser application (Fig. 4D).

DISCUSSION

This study clearly shows that subsurface vessels can be visualized and blood flow measured by CDOCT and that the response of these vessels to various hemostatic treatment modalities can be readily demonstrated. The results confirm many well-established tenants of endoscopic treatment of bleeding GI lesions, but they also raise some questions with respect to the manner in which hemostatic modalities and devices are applied. In addition to its use in the experimental setting, the results suggest that CDOCT might be useful clinically as a method to refine hemostatic techniques with the potential to improve outcomes for patients with GI bleeding.

Four different therapeutic interventions that are used in humans in the endoscopic treatment of bleeding ulcers were chosen intentionally: epineph-
rine injection; injection of sclerosant; heat probe thermal contact coagulation; and laser photocoagulation. It is evident from our study that blood flow may not completely cease after epinephrine injection and that part of the effect may be transient. After epinephrine injection, there was initial absence of detectable blood flow by CDOCT (at least for the first hour). Interestingly, there was a resumption of minimal blood flow at 3.0 and 4.5 hours. However, the peak flow velocity had diminished by more than 50% when compared with pretreatment values. Despite the reduction in blood flow, the subsurface blood vessels appeared histologically normal and there was no evidence of intravascular thrombosis or adjacent inflammatory response (Fig. 2C). This is in agreement with the previous findings of Rutgeerts et al.,\textsuperscript{21} that submucosal injection of epinephrine does not induce significant tissue injury, nor does it result in vessel thrombosis. This observation may have important clinical implications regarding the effectiveness of epinephrine injection alone at securing long-term hemostasis in bleeding ulcers. Our experimental findings are supported by the clinical demonstration that despite equivalent efficacy at halting the primary bleeding, epinephrine injection alone is less effective than combination treatment with thermal therapy, in terms of securing long-term hemostasis in ulcers with active spurting bleeding.\textsuperscript{1}

In contrast to epinephrine injection, sclerosant injection, heat probe, and laser treatments resulted in the immediate and complete cessation of detectable blood flow with clear, histologic evidence of vascular injury. Unexpectedly, CDOCT demonstrated that blood flow ceased in response to the heat probe before there was any visual evidence of significant surface injury. The tissue was not histologically sectioned after the initial application of 20 J, but it is worth speculating whether this may have shown early evidence of intravascular thrombosis and tissue coagulation in response to the limited application of heat alone. This finding suggests that a reevaluation of treatment endpoints in thermal contact therapy may be required. For instance, the recommended endpoint in the thermal contact treatment of the nonbleeding visible vessel is complete flattening.\textsuperscript{22} Often, the treatment creates a charred ulcer, which is much larger than the original visi-
ble vessel. This raises the possibility that some of the risk of recurrent bleeding may, in part, be due to the endoscopic thermal treatment itself. Perhaps the goal of endoscopic therapy should be the elimination of detectable blood flow in the bleeding blood vessel and not complete ablation of major endoscopic surface stigmata. This paradigm shift would require further, intensive research with modern technologies able to accurately monitor blood flow in subsurface blood vessels (such as CD-OCT) by using both animal models and in prospective human clinical studies.

The ability to obtain high-resolution real time endoscopic images of the human GI tract with OCT methodology in vivo has recently been demonstrated.23-28 Currently, an investigation is underway of the possibility of incorporating color Doppler technology into our human in vivo OCT system. The ability of CD-OCT to closely monitor the dynamics of blood flow in this animal model demonstrates its potential use in evaluating new and existing methods of hemostatic therapy. As an imaging modality, CD-OCT may be useful in the endoscopic assessment of acutely bleeding ulcers. It may be possible to locate and map the bleeding submucosal blood vessel with a degree of precision that would allow less aggressive, but more effective targeting of endoscopic therapy. By using continuous CD-OCT monitoring, the endoscopist may be able to more accurately titrate the amount of thermal energy used, based on detectable blood flow. These factors may potentially result in a better patient outcome and a lower risk of perforation or transmural injury after endoscopic therapy. Moreover, it may provide an estimate of vessel diameter and thereby alert the endoscopist to the presence of a large vessel that would not be amenable to endoscopic treatment and which carries heightened risk for uncontrollable hemorrhage.

There are some limitations to CD-OCT. For instance, imaging with the currently available OCT equipment is limited to a depth of 1 to 2 mm beneath the tissue surface. This may prove to be problematic if the blood vessel of interest is located deeper in the tissue. Nevertheless, in published studies with endoscopic (nonimaging) Doppler US in acutely bleeding gastric and duodenal ulcers, the US-scanned depth has been limited to 1 mm beneath the ulcer base.4,29

There are potential advantages of OCT or CD-OCT over high-frequency endoscopic US. First, imaging Doppler US is not currently available in probe design, unlike in vivo catheter probe-based endoscopic OCT in humans. Second, OCT has a much higher spatial resolution in clinical use (10-20 μm) than currently available high-frequency (10-30 MHz) US equipment (~100 μm). Third, unlike US, OCT does not require tissue contact or liquid medium (acoustic coupling) for optimal imaging. Fourth, the use of fiberoptics with OCT may allow for more flexible imaging in the design of the endoscope imaging tip.

**DISCLOSURE**

Drs. M. V. Sivak, Jr., and J. A. Izatt have a proprietary interest in Doppler OCT technology.

**REFERENCES**

15. Kulkarni MD, van Leeuwen TG, Yazdanfar S, Izatt JA. Velocity-estimation accuracy and frame-rate limitations in