Measurement of mucosal capillary hemoglobin oxygen saturation in the colon by reflectance spectrophotometry

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Background: **Advances in optical and computer technology have enabled the development of a device that uses white-light reflectance spectrophotometry to measure capillary hemoglobin saturation in intestinal mucosa during colonoscopy.**
Methods: **Studies were performed with the colon oximeter in anesthetized animals and patients undergoing colonoscopy.**
Results: **Mean (SD) mucosal hemoglobin saturation in the normal colon was 72% (3.5%). In an animal model, ischemia induced by arterial ligation and hypoxemia via hypoxic ventilation each resulted in a decrease of over 40% in the mucosal saturation. In patients with colon polyps, ischemia induced by epinephrine injection, stalk ligation with a loop, or clipping of the polyp stalk each resulted in a decrease of over 40% in the mucosal saturation ($p < 0.02$). In contrast, saline solution injection did not decrease the mucosal saturation.**
Conclusions: **A novel device for measuring capillary hemoglobin saturation in intestinal mucosa during colonoscopy is capable of providing reproducible measurements in normal patients and clearly detects dramatic decreases in saturation with ischemic and hypoxic insults. (Gastrointest Endosc 2003;57:492-7.)**

The colon is the most common site of intestinal ischemia.[1] Ischemic colitis commonly occurs during low flow states, such as septic shock, and as a complication of aortic surgery.[1] There has been a spate of cases of ischemic colitis attributed to administration of the 5-HT3 antagonist alosetron.[2,3] The actual incidence of ischemic colitis may not be known because of the difficulty of making the diagnosis in all but the most severe cases.[1,2] Currently, the diagnosis of colonic ischemia is based on clinical suspicion: often, there is a history of a potentially precipitating event followed by abdominal pain and bloody diarrhea. Radiologic studies, such as plain x-ray films, contrast radiography of the colon, and CT are all limited by poor sensitivity and specificity in early or mild cases.[1] Angiography demonstrates an arterial embolus or thrombus in some cases, but the majority of ischemic episodes are nonthrombotic.[4] Frankly necrotic bowel may be seen at colonoscopy in extremely severe cases, but more commonly there are less specific findings such as erythema or ulcerations; biopsy specimens are frequently obtained, but are usually not helpful.[1] An objective method for measuring colonic perfusion would be useful, both as a research technique and potentially as a clinical method of diagnosis.

Reflectance spectrophotometry, an optical technique for the assessment of GI mucosal perfusion, was first described by Sato et al.[5] in 1979. Although imaging methods such as optical coherence tomography and high frequency US can visualize arterial and venous vessels, and techniques such as laser Doppler and speckle can provide information about the velocity of blood in the tissue, reflectance spectrophotometry directly measures the oxygen saturation of capillary hemoglobin. Typical configurations use an endoscopic catheter that contains optical fibers for the delivery of a calibrated white light to the mucosa and for recovery of reflected and scattered light for analysis by a spectrophotometer.[6,7] Early devices were limited by their capability to analyze the recovered light at only 3 wavelength ranges.[8] Advances in optical equipment and computer technology now allow measurements at hundreds of wavelengths and analysis in real-time by sophisticated software. By analyzing the recovered signal, it is possible to obtain quantitative measurements of the oxygen saturation of hemoglobin in the mucosa.[7] In contrast to pulse oximetry, which measures arterial hemoglobin saturation, mucosal reflectance oximetry measures hemoglobin within capillary erythrocytes, and to a lesser extent in small arterioles and venules, and therefore this

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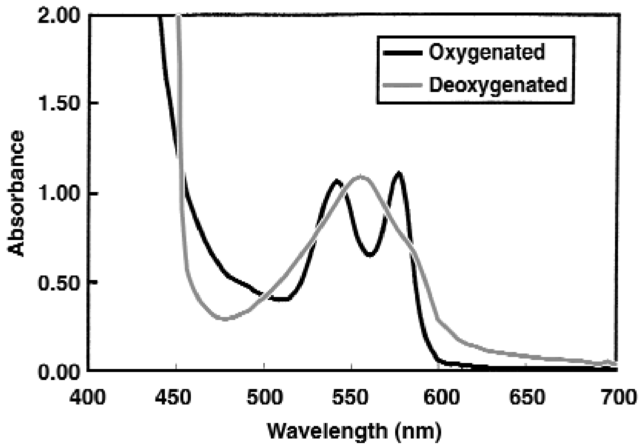

Figure 1. Spectra of oxygenated and deoxygenated hemoglobin.

measurement reflects the perfusion status of the GI mucosa.[9] Reflectance spectrophotometry thus has the potential to be a useful, relatively noninvasive technique for measuring gut perfusion.

METHODS AND MATERIALS

Endoximeter device

Endoscopic probes were constructed by threading two 200-micron optical fibers into a flexible plastic catheter. One fiber was coupled to a white filament lamp and the other to a spectrophotometer (unpublished observation). The spectrophotometer output was analyzed in real time using a portable computer with custom designed software. The power output of the optical fiber was 0.8 mW, which is substantially below maximal permissible exposure standards for tissue.

The device was calibrated before each use with a white reference consisting of a standard 2% fat emulsion product used for parenteral nutrition (Intralipid, Baxter Healthcare, Deerfield, Ill.) in saline solution. Spectra of 100% oxygenated and 100% deoxygenated hemoglobin were obtained by direct measurement (oxygenated) and from published reports (deoxygenated).[10] To account for differences caused by the angle of approach of the probe to the mucosa, the variable distance from the mucosa, and the scattering characteristics of the mucosa, the analysis software performs a least-squares fit of a first order polynomial and oxy/deoxyhemoglobin spectra to the measured spectrum, which eliminates much of the effect of scattering on the measured spectrum. The fitting is done at 520 nm to 600 nm, an area of the hemoglobin spectrum in which there are substantial differences between the oxy and deoxy forms (Fig. 1), and for which the penetration of the reflected light is limited to a depth of approximately 1 mm. Based on the best-fit algorithm, the relative concentrations of oxy- and deoxy-hemoglobin are calculated.

Measurement technique

After maneuvering the colonoscope to the region of interest, the mucosa was lavaged with water to wash away bile and stool (which are optically active and can influence the measurement). The probe was then passed through the colonoscope accessory channel and positioned a few millimeters away from the mucosal surface. The endoscope light was briefly turned off and the measurement was performed. Each measurement required 300 milliseconds. Five separate measurements were obtained and the results averaged. The angle of the probe, distance from the mucosa, and motion of the probe and subject did not substantially affect readings (data not shown).

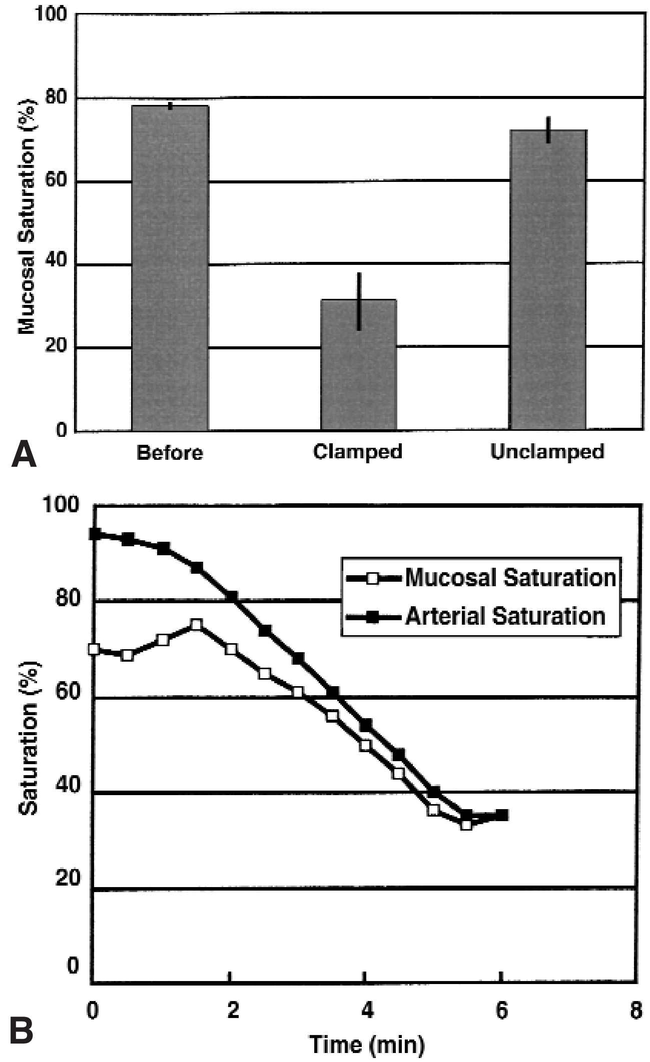

Figure 2. A, Response of porcine sigmoid mucosal saturation to clamping of arterial supply. Vertical error bars indicate ±1 SEM. **B,** Response of porcine sigmoid mucosal saturation and pulse oximetry (auricular artery) to hypoxemia induced by interruption of oxygen flow to ventilator.

Animal studies

Animal studies were performed in a dedicated animal facility with institutional review board approval. After induction by an intramuscular injection of ketamine (9 mg/kg) and diazepam (0.25 mg/kg), 40-kg farm pigs were anesthetized with isoflurane and nitrous oxide titrated to

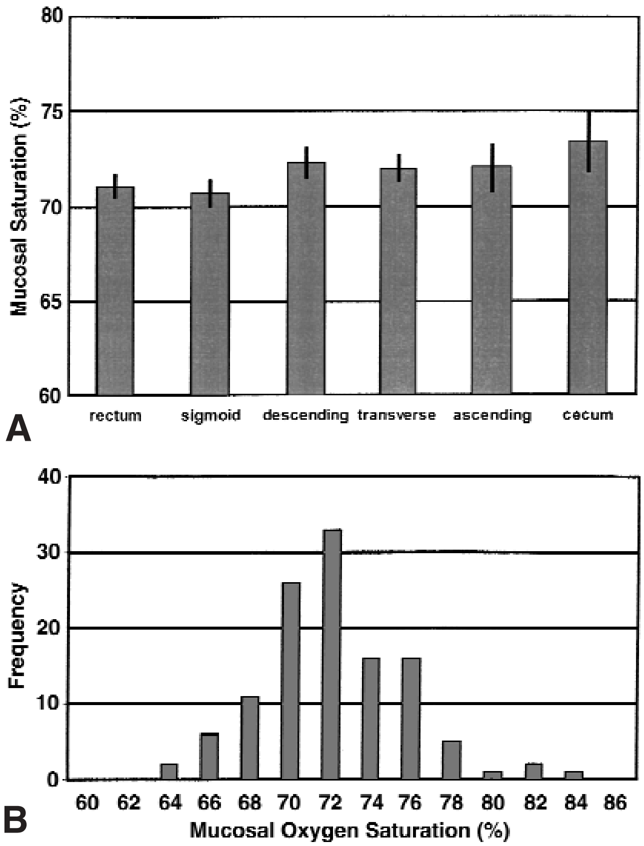

Figure 3. A, Mucosal oxygenation by region in normal colon. Vertical error bars indicate ±1 SEM. **B,** Histogram of mucosal oxygen saturation measurements in normal colon.

achieve and maintain deep anesthesia. The colon was cleansed with large-volume water enemas. Hypoxic measurements were performed while the animals were under deep general anesthesia by halting oxygen flow into the ventilator system. Segmental colonic ischemia was induced by ligating the arteries supplying the left colon (including the collateral arcades).

Human studies

Human studies were performed with informed patient consent under a protocol approved by the institutional review board of our hospital. Patients were referred for either colonoscopy or sigmoidoscopy for a variety of indications, most commonly colon cancer screening or surveillance for polyps. Patients were classified as normal controls if they had no history of inflammatory bowel disease, abdominal or pelvic radiation, or partial colectomy. Patients were excluded if referred for suspected colonic ischemia or active colitis, or were hypotensive (systolic blood pressure <90 mm Hg). Measurements were not performed in or adjacent to diverticula or during colonic contractions. A total of 119 measurements in various segments of the colon were obtained in 40 normal control patients, all from normal-appearing mucosa. During many of the procedures, measurements were not obtained in all segments of the colon because of time constraints (at the discretion of the endoscopist).

Table 1. Characteristics of patients

Characteristic	Percent (N = 40)
Gender	
Male	90
Female	10
Age	
<50	7.5
50-59	30
60-69	25
70-79	27.5
>79	10
Procedure	
Sigmoidoscopy	7.5
Colonoscopy	92.5
Indication	
Screening	10
Polyp on screening examination	32.5
History of polyps	30
Hematochezia	10
Occult blood in stool	5
Other*	12.5

*Other indications for endoscopy include one each of constipation, diarrhea, anemia, abdominal pain, and rectal cancer.

In the studies of polyps, saline solution injection, epinephrine injection, and application of clips or endoscopic loops to stalks were performed as needed for polypectomy of large or sessile lesions. Polyp oxygenation values are shown with error bars representing ±1 standard error of the mean (SEM). All of the polyp experiments were repeated at least once with similar results; representative cases are shown.

RESULTS

The change in mucosal oxygen saturation in response to ligation of the vascular supply to the porcine sigmoid colon is demonstrated in Figure 2A. The measured tissue oxygen saturation declined from 78% to 31% with this maneuver and returned nearly to baseline 1 minute after release of the ligatures. The results of hypoxic ventilation in the porcine model are demonstrated in Figure 2B. Within approximately 1 minute of discontinuing oxygen delivery to the ventilator, the pulse oximeter demonstrated a slight decline in arterial oxygen saturation and a further progressive decrease over the next 5 minutes. Sigmoid colon tissue oxygenation transiently increased slightly during the first 90 seconds, and then steadily decreased over the next 5 minutes to a value of 35%.

Demographic data for the 40 normal control patients are presented in Table 1 (the high proportion of men reflects the population served by the main facility used for the study, a Veteran Affairs hospital). The mean (SD) mucosal oxygen saturation was 72% (3.5%). The mean measured mucosal oxygen saturation by colon region ranged from 71% in

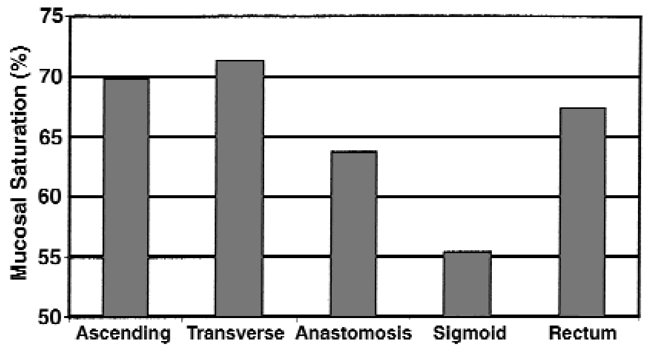

Figure 4. Mucosal saturation measurements in 61-year-old man who had undergone resection of 15 cm of sigmoid/descending colon with removal of inferior mesenteric artery.

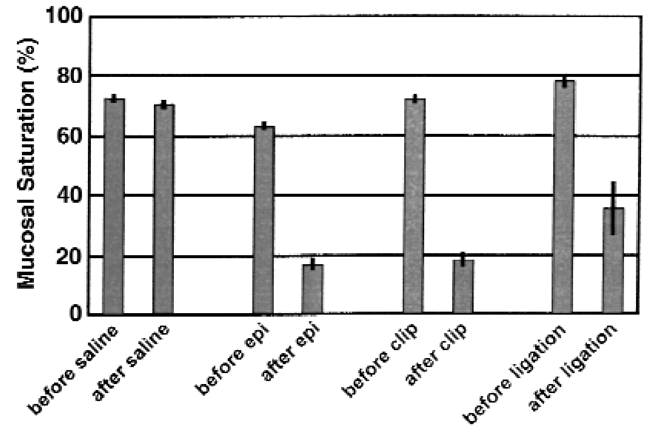

Figure 5. The effect of ischemic interventions on mucosal oxygenation of polyps. Shown are saline solution injection (control), epinephrine injection, clipping of stalk, and ligation of stalk. Vertical error bars indicate ±1 SEM.

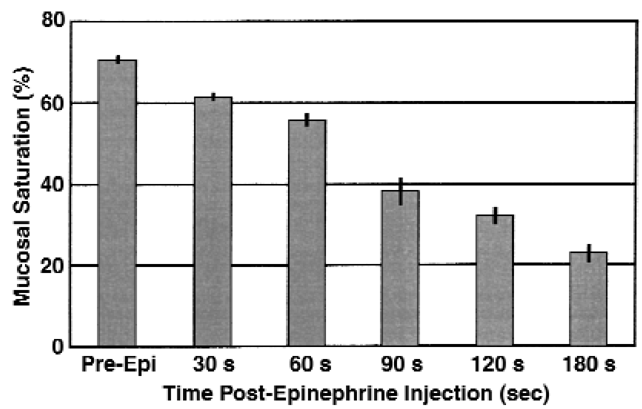

Figure 6. Time course of mucosal ischemic response to epinephrine injection. Vertical error bars indicate ±1 SEM.

the rectum and sigmoid colon to 73% in the cecum (F = 1.1; NS), as shown in Figure 3A. A histogram of all measurements is shown in Figure 3B.

One patient had significantly different oxygenation values in different regions of the colon (Fig. 4). This patient was an asymptomatic 61-year-old man who had undergone a partial colectomy 5 years previously for a T3N1 adenocarcinoma. During the operation, 15 cm of sigmoid/descending colon were resected, and the inferior mesenteric artery was removed. The surveillance colonoscopy demonstrated an intact anastomosis 25 cm from the anal verge, with approximately 10 cm of normal-appearing sigmoid colon distal to the anastomosis. The measured mucosal saturation in the remaining portion of the sigmoid colon was 55%.

The effects on the measured tissue oxygen saturation of interventional endoscopic manipulations known to cause ischemia are illustrated in Figures 5 and 6.

Clips are commonly applied at endoscopy to either prevent bleeding or treat bleeding lesions in the GI tract. A decrease in oxygenation of a large polyp from 72% to 18% in response to clip application is illustrated in Figure 5. Application of an endoscopic loop to the stalk of a polyp caused a decrease in tissue oxygenation from 78% to 36%.

Submucosal injection of saline solution and of a dilute solution of epinephrine are commonly used during polypectomy—saline to separate the mucosal lesion from the underlying submucosa for safer resection and epinephrine to lift the mucosa and decrease bleeding. Injection of epinephrine induces detectable local ischemia, whereas injection of saline solution does not (Fig 5).

The time course of the ischemic response to epinephrine injection is shown in Figure 6. Within 30 seconds after epinephrine injection, tissue oxygenation decreased from 71% to 61%. This was followed by a steady decrease to 23% after 3 minutes.

DISCUSSION

The present study demonstrates that consistent, reproducible measurements of mucosal oxygen saturation can be obtained during colonoscopy. The average mucosal saturation measured in control patients was 72% (3.5%). No statistically significant differences were observed among the various segments of colon, except in one asymptomatic patient who had undergone resection of 15 cm of sigmoid/descending colon with removal of the inferior mesenteric artery 5 years previously. In this patient, the mucosal saturation was 55% in the remaining portion of the sigmoid colon (Fig. 4), approximately 5 standard deviations below normal and substantially lower then any measurement in the 40 control patients (Fig. 3; $p < 0.05$). This particular patient had a normal mucosal saturation in the ascending and transverse colon (respectively, 70% and 71%; NS) and a slightly decreased saturation in the rectum

(67%; NS). These observations are consistent with detection of stable, but chronically decreased perfusion of the residual sigmoid colon after removal of the inferior mesenteric artery, and intact perfusion in the ascending and midtransverse colon (supplied by superior mesenteric artery) and rectum (multiple arterial sources). At least in this patient, this level of reduced perfusion is not sufficient to cause clinical manifestations.

The studies in animals demonstrated that severe hypoxemic or ischemic insults reduced sigmoid mucosal oxygenation by 40% or more. Studies of epinephrine injection and clip application or ligation of polyp stalks in patients demonstrated that these maneuvers also result in dramatic decreases in tissue oxygenation of the involved areas. These observations document that the reflectance spectrophotometry device used in the present study performs as expected in experimentally controlled conditions and suggest that it has the potential to provide meaningful measurements in clinical diseases.

Reflectance spectrophotometry has been used in research to measure GI mucosal perfusion since the late 1970s, after the pioneering work of Chance et al.,[11] who devised a flexible light guide for spectroscopy. With the present technique, a small superficial tissue volume is sampled, typically a spot approximately 2 mm in diameter with a depth of under 1 millimeter.[7] The quantity measured is the relative concentration of oxygenated versus deoxygenated hemoglobin in the tissue volume sampled. Because most of the hemoglobin is contained in erythrocytes within capillaries, and to a lesser extent small arterioles and venules, the measured hemoglobin oxygen saturation reflects the perfusion status of the mucosa.[9] Earlier studies were performed in the stomach and duodenum because these areas were accessible without bowel preparation and were of particular interest with respect to the study of septic shock and portal hypertension.[6,8,12,13] The technique was found to correlate well under experimental conditions with other research methods for measuring gut perfusion, such as hydrogen gas clearance.[8] However, reflectance spectrophotometry remained an experimental technique with limited applications because older instruments were not robust enough to provide reliable measurements in clinical practice. Recent advances in instrumentation and signal processing capabilities have made it possible to construct instruments, such as ours, that perform measurements at hundreds of wavelengths and use fitting algorithms that can correct for uneven spectral baselines and provide rapid, reliable measurements. The apparatus described in this report is now available as a self-contained portable unit that can be operated in any endoscopy unit. Our expectation is that a device application will be submitted to the United States Food and Drug Administration in 2003.

A suitable device for measurement of colonic perfusion will allow objective evaluation of disorders such as ischemic colitis for which prior studies have been limited by current diagnostic techniques.[1] In addition, the postulated role of ischemia in disorders such as chronic radiation proctitis,[14] toxic megacolon, and various infections[1] can be examined in rigorous scientific studies. With regard to the recent spate of cases of ischemic colitis attributed to use of alosetron, it is possible that a technique such as reflectance spectrophotometry could be used to identify patients in whom ischemia develops early in their course, rather than waiting until fulminant disease becomes clinically manifest. It is our belief that tissue oxygenation is a fundamental physiologic variable and that the ability to measure it simply and relatively noninvasively will lend itself to creative and useful applications.

DISCLOSURE

Dr. Benaron holds equity in and receives financial compensation from Spectros Corporation, an NIH- and NCI-supported medical device concern, which is developing a commercial device for measuring tissue capillary oxygenation.

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