Optical Microangiography: High-Definition Magnification Colonoscopy with Narrow Band Imaging (NBI) for Visualizing Mucosal Capillaries and Red Blood Cells in the Large Intestine

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Background/Aims: Recent advances in zoom endoscopy have enabled the subepithelial capillary network (SECN) in different organs of the gastrointestinal tract to be visualized. *Ex vivo* studies have suggested that the SECN demonstrates a honeycomb-like structure in the large intestine, but this has not yet been visualized *in vivo*. The high clarity and resolution of narrow-band imaging (NBI) may allow visualization at the single red-blood-cell (RBC) level and more accurate visualization of the SECN. We investigated whether high-definition magnification colonoscopy with NBI is useful for visualizing capillaries and RBCs in the large intestine. **Methods:** Sixteen patients with bowel symptoms undergoing routine colonoscopy with normal findings in a tertiary referral academic gastroenterology and endoscopy unit were included in the study. Total colonoscopies were performed using a high-definition magnification colonoscope (CF-H260AZI, Olympus, Tokyo) and a prototype high-definition electronic endoscopy system capable of NBI. Each part of the large intestine (cecum, ascending, transverse, descending, and sigmoid colon, and rectum) was observed at the maximum magnification with white-light imaging (WLI) and NBI. The normal honeycomb-like SECN and RBC movement by high-definition magnification colonoscopy with either WLI or NBI was prospectively successfully visualized for each part of the large intestine. **Results:** In all subjects, high-definition magnification colonoscopy with NBI allowed the visualization of a honeycomb-like SECN together with RBC movement in each segment of the large intestine except for the rectum. In contrast, with WLI alone, nei-

ther this SECN structure nor RBC movement could be detected. **Conclusions:** High-definition magnification colonoscopy with NBI could be a new optical method for facilitating noninvasive investigations of both the microvascular architecture and microcirculation without the need for contrast materials. (*Gut and Liver* 2008; 2:14-18)

Key Words: Optical; colonoscopy; Capillary; Red blood cell; Large intestine

INTRODUCTION

Mucosal blood flow and normal microvascular architecture can be disturbed in various gastrointestinal diseases including neoplasia and inflammatory conditions. Endoscopic visualization of normal mucosal microvascular architecture and blood flow is important in order to investigate these changes. With regard to the large intestine, there have been reports that had demonstrated normal microvascular architecture as a honeycomb-like subepithelial capillary network (SECN) pattern *ex vivo*,^{1,2} however, optical magnification endoscopy with ordinary white light imaging (WLI) alone can not visualize such a honeycomb-like SECN pattern or microcirculation. Recently, a new optical technique called "narrow-band imaging (NBI)" which is designed to help specifically the visualization of superficial microvasculature has been developed.³ If we apply NBI to high-definition magnification endoscopy (ME) which has enough resolution power to

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dissect capillary or red blood cell (RBC) in the large intestine, we may be able to visualize both normal microvascular architecture and RBC movement. According to this hypothesis, we tested whether or not high definition ME with NBI could constantly visualize both normal microvascular architecture and RBC movement *in vivo*.

MATERIALS AND METHODS

A high-definition magnification colonoscope (CF-H260AZL, Olympus-Keymed, United Kingdom) with a prototype hi-definition electronic endoscopy system that incorporates NBI function (Hyperpro, Olympus-Keymed, United Kingdom) was used in this study. This colonoscope can magnify optically up to 150-fold through the use of a motorized zoom lens fitted to the end of the colonoscope and activated via lever in the scope head. Before the colonoscopy, a soft black hood (MH189, Olympus, Japan) was mounted on the tip of the endoscope to enable the endoscopist to fix the focal distance at 3 mm between the tip of the colonoscope zoom lens and the mucosal surface, the point at which maximal magnification of the endoscopic image can be obtained.

The maximal resolution power of the magnification colonoscope was measured under laboratory condition as previously described.⁴ Briefly, a resolution chart (Toppan test chart No. 1, Toppan, Japan) was used on which black lines of various known widths and lengths are displayed. The chart was placed on a stage, arranged perpendicular to the axis of the insertion tube of the endoscope. At maximal magnification, the minimal width (in μm) of the black lines that could be separated visually on the endoscopic high-definition monitor was determined and taken as the maximal resolution power of the magnifying endoscopic system.

1. Materials

Sixteen consecutive patients with bowel symptoms undergoing routine colonoscopy that was normal were included in the study. Patients who had a pathology identified anywhere in the colon were excluded. The median age (range) of the patients was 41 (25-80) years old, 9 were male and 7 female. All the biopsy specimens were reported by a gastrointestinal pathologist (PK) who was blinded to endoscopic findings and all the biopsy specimens were confirmed as having no pathological findings. The protocol was approved by the medical ethical committee of Nottingham University Hospital and written informed consent was obtained from all the patients before the procedure.

2. Endoscopic procedure

All the endoscopy procedures were performed by three endoscopists (KY, GA, KR) who are highly experienced in magnification colonoscopy. The scope was inserted into the cecum with WLI, and then the patients were alternatively assigned to the two different procedures, that is, either WLI or NBI was firstly used to observe the cecum. For example, if the white light is selected as the first imaging modality, the cecal mucosa was investigated carefully by non-magnified endoscopy with WLI first. When it shows normal appearance of the mucosa, the tip of the hood is allowed to contact perpendicularly to that part of the mucosa immediately after zooming up to the maximal magnification, and then magnified endoscopy image with WLI was obtained. Secondly, after switching WLI to NBI, ME image with NBI was obtained at the same magnification rate. Holding with NBI mode, the next segment, namely, the ascending colon was examined by non-magnified observation with NBI followed by ME with NBI and subsequently by ME with WLI. Accordingly, six segments of the large intestine (cecum, ascending, transverse, descending, sigmoid colons and rectum) were consecutively observed at maximal magnification alternatively with WLI and with NBI. This method using hood attachment enables the endoscopist to obtain magnified endoscopic images with both WLI and NBI at exactly the same maximal magnification rate. One target biopsy was taken from each of the corresponding part of the mucosa which was observed by ME. After the examination, whether or not the normal honeycomb-like SECN pattern and RBC movement are visible within capillaries in the six segments within the large intestine by magnification with both WLI and NBI were recorded in the medical notes on the hospital database system. All procedures were video recorded and digital pictures taken. 192 paired digital images (96 WLI, 96 NBI) from 16 patients taken from the 6 segments of the colon and rectum were chosen to be reviewed. The images and video clips were jointly reviewed by KY and KR.

RESULTS

1. Resolution power of the high definition magnification colonoscope

At maximal magnification, the smallest line width separable on the resolution chart was $7.016 \mu\text{m}$; lines $6.250 \mu\text{m}$ in width could not be separated. Accordingly, the resolution power of this high-definition magnifying colonoscope was determined to be $7.1 \mu\text{m}$.

2. The visibility of normal honeycomb-like SECN pattern and RBC movement

In all 16 patients, high definition ME with NBI could constantly visualize a honeycomb-like SECN pattern in each part of the large intestine (Fig. 1, 2) except for the rectum (Fig. 3). In the rectum, high-definition ME with NBI could not uniformly visualize a honeycomb-like SECN pattern, that is, 5 out of 16 patients demonstrated a honeycomb-like SECN pattern, while the rest of 11 out of 16 patients showed incomplete honeycomb-like SECN pattern where single loop of capillary did not show completely-closed polygonal shape. With regard to RBC move-

ment, we successfully observed RBC movement within subepithelial capillaries in all the parts of all the patients by ME with NBI. In contrast, by ME with WLI, neither honeycomb-like SECN pattern nor RBC movement could be visualized.

DISCUSSION

Recent advances in ME has enabled endoscopist to observe the organ-specific mucosal subepithelial microvascular architecture in the gut mucosa.⁵⁻⁸ With regard to the esophagus, stomach, and duodenum, such normal microvascular as small as capillary, which is the smallest

Fig. 1. Magnified endoscopic findings of the cecal mucosa as visualized by high-definition magnification colonoscopy. (A) With WLI alone, a honeycomb-like SECN pattern is not noted clearly. (B) When we change WLI to NBI, a typical honeycomb-like SECN pattern is clearly visualized. Each polygonal capillary loop surrounds a crypt opening and forms honeycomb-like network beneath the epithelium.

Fig. 2. Magnified endoscopic findings of the descending colon as visualized by high-definition magnification colonoscopy. (A) When we observe the mucosa at maximal magnification with NBI first, a honeycomb-like SECN pattern is evident. (B) When we change NBI to WLI, however, a honeycomb-like SECN pattern has disappeared.

Fig. 3. Magnified endoscopic findings of the rectum as visualized by high-definition magnification colonoscopy. (A) Magnification endoscopy with WLI of the rectum does not reveal any subepithelial capillaries. (B) Magnification endoscopy with NBI of the rectum reveals an incomplete honeycomb-like SECN structure, with each capillary loop appearing as a not-closed polygon.

unit of the human vessels, can be well visualized by high resolution optical ME with WLI alone.⁵⁻⁸ However, although the normal honeycomb-like SECN pattern has been well demonstrated with dissecting microscopy,¹ and scanning electron microscopy using corrosion casts *ex vivo*,² optical ME with WLI could not visualize normal microvascular architecture.

Our study was prompted by the observation that a honeycomb like SECN, and detectable RBC movement were evident under NBI. We therefore set out to study this systematically and compare visualization with that achieved under WLI. High-definition magnifying endoscopy had a 7.1 micrometer resolution in this study but the improved precision of imaging achieved with NBI was needed before these findings could be seen clearly. From the optical point of view, since NBI utilizes an optical filter with narrow bandwidths of two different central wavelengths (415 and 540 nanometers), the penetration of the lights through NBI filter is very shallow compared with ordinary white light and the absorption of hemoglobin pigment has two peaks in such both two wavelengths (415 and 540 nm).³ This optical design of NBI has advantages to that of ordinary WLI in visualizing the capillary together with RBC movement which is located just underneath the mucosa.² From the technical point of view, it is suggested that the use of hood to fix the distance between the tip of the scope and the mucosal surface is mandatory in order to constantly observe the precise mucosal microvascular architecture and RBC movement using the maximal resolution power (7.1 μ m) of the high-definition magnification colonoscope. We stress that we cannot constantly observe the fine microvessel as

small as capillary even with NBI if we do not use such hood technique. Several feasibility studies using NBI endoscopy is now accumulating,⁹⁻¹² however, no study as yet has demonstrated real advantages of NBI to WLI for visualizing mucosal microvascular architecture or RBC movement. This is the first report to demonstrate such an advantage of NBI in clinical practice that can be applied for future research studies.

Angiogenesis or new vessel formation plays a key role in the pathogenesis of colorectal neoplasia.¹³ Experimental studies have shown that it may occur as early as the transition from hyperplasia to neoplasia.¹⁴ Recently Sano et al reported that a meshed capillary vessel which is detected by ME with NBI is a reliable marker for differentiating neoplasia from non-neoplasia.¹⁵ However, this study was not designed to systematically define the normal microvascular architecture in the colon. Furthermore, since changes in microvasculature and microvascular density occur in the process of inflammation, detecting such changes can be useful in the grading, assessment of response to treatment, and the diagnosis of remission or relapse of inflammatory bowel diseases.

In vivo, confocal laser endoscopy was the only modality which can visualize normal SECN in the large intestine.¹⁶ However, this technique needs special equipment other than ordinary optical endoscopy system. Furthermore, in order to visualize mucosal microvascular architecture, it is mandatory to inject fluorescent materials to the patients which in itself is cumbersome and can cause toxic or allergic side-effects. In conclusion, high definition ME with NBI using a hood attachment could be a new optical method that facilitates non-invasive investigation for both

microvascular architecture and microcirculation without any contrast materials. The technique and the findings described here could be used as a benchmark when we study the endoscopic pathology in the large intestine.

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