Confocal Laser Endomicroscopy of the Colon

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Abstract

Confocal laser endomicroscopy (CLE) has been recently proposed as a new technique that allows in vivo histologic assessment of mucosa during endoscopy. The most commonly used contrast agents are acriflavine hydrochloride and fluorescein sodium. For colon pathology assessment, the administration of fluorescein intravenously produces a strong staining of both surface epithelium and deeper layers of lamina propria.

Confocal laser endomicroscopy is a feasible method to diagnose colon cancer in vivo. Furthermore, confirmation of neoplastic changes using CLE during colonoscopy may lead to major improvements in the clinical management of the patients with inflammatory bowel disease. Biopsies can be limited to targeted sampling of relevant lesions.

Confocal laser endomicroscopy will certainly play an important diagnostic role during gastrointestinal endoscopy in the future, enabling the elimination of the diagnostic delay associated with conventional biopsy preparation and processing.

Keywords


Introduction

Magnifying, high-definition endoscopy systems, used frequently in conjunction with chromoendoscopy, narrow band imaging (NBI) or autofluorescence endoscopy, enable detailed surface analysis which allows the identification of suspicious areas within the gut [1]. Even so, microscopic tissue evaluation is considered the gold standard for the final diagnosis of most diseases in gastroenterology [2]. Nowadays, the endoscopist is challenged by many mucosal details, and all of these new imaging modalities require adequate training, especially by pathologists [3].

Confocal laser endomicroscopy (CLE) has been recently proposed as a new technique that allows in vivo histologic assessment of mucosa during ongoing endoscopy, retrieving simultaneous endoscopic and endomicroscopic information and images in real-time. The immediate question that arose was: do we still need the pathologist for diagnosis? [3]. Confocal laser endomicroscopy allows imaging of the mucosal layer, including epithelial cells and the lamina propria. The targets of endomicroscopic examination for an accurate diagnosis can be the cells, vascular structures, and/or tissue patterns [4].

Furthermore, if an immediate diagnosis cannot be obtained by endomicroscopy, the same technique offers the possibility of targeted biopsies. The pathologist will receive fewer biopsy specimens but will find more specific tissue because a greater number of intelligent biopsy specimens containing target tissue are provided by the endoscopist [3].

Recently, the potential role of CLE has been explored in different pathologic conditions of the gastrointestinal tract, such as Barrett’s esophagus, premalignant and malignant lesions of the stomach [5] and colon [6].

Principle and technique

The principle of CLE is based on the integration of a confocal laser microscope into the conventional endoscope, allowing a simultaneous endoscopic and endomicroscopic examination in real-time. The outer diameter of the endoscope is 12.8 mm with a working channel of 2.8 mm. During the procedure, a laser device delivers an excitation wavelength of 488 nm that is focused onto a distinct imaging plane within the tissue. The confocal image data are captured at scanning rates of 0.8 frames/s (1024x1024 pixels) or 1.6 frames/s (1024x512 pixels). The optical slice thickness is 7 µm, with a lateral resolution of 0.7 mm (field of view...
475x475 μm). The range of depth is 0-250 μm below the surface layer [7, 8].

**Contrast agents**

Potentially appropriate contrast agents used during CLE are represented by fluorescein, acriflavine, tetracycline, or cresyl violet. The most commonly used are acriflavine hydrochloride (0.05% in saline, topical use only) and fluorescein sodium (5 ml of a 10% solution, intravenous administration) [7]. Fluorescein is a slightly acidic, hydrophilic dye with nonspecific staining properties. It is currently used by ophthalmologists for fluorescence angiography. Within seconds of intravenous administration, it binds extensively to serum albumin in the blood, establishing a stable distribution throughout the entire mucosa and providing strong contrast. Only the free molecules are rapidly cleared from the plasma by the liver and excreted by the kidneys. Structures observed with fluorescein dye include colonic pit patterns, surface epithelial cells, connective tissue matrix of the lamina propria, blood vessels and red blood cells. Cell nuclei are only rarely distinguished because fluorescein only occasionally crosses the lipid membranes and does not bind to nucleic acids [8]. However, nuclei are of special interest in assessing low- and high-grade dysplasia. For this reason, local application of acriflavine may help to overcome this problem [3]. Acriflavine was developed as an antifungal agent to be used in local antiseptic solutions. Topically administrated, it passes cell membranes and displays a strong specificity for labeling acidic constituents, but acriflavine does not result in the staining of the deeper layers of the gastrointestinal mucosa. It predominantly stains the nuclei of the surface epithelium and enables differentiation of columnar epithelial cells, goblet cells, and different normal and pathologic patterns [8].

**Artifacts**

Gastrointestinal peristalsis and patient breathing can cause movement artifacts (Fig. 1) in some images, although this situation is more prominent in upper-GI imaging, particularly in the vicinity of the cardia. Suction of the superficial mucosa via the working channel can solve this problem because it immobilizes the tip of the endomicroscope on the examined surface [8]. Other sources of CLE image artifacts includes inappropriate contact of the endomicroscope imaging window with the mucosal surface resulting in dark spots or circles caused by mucus, fecal content, and air bubbles at the tissue interface with the distal tip of the scope (Fig. 2).

**Colon patology**

**Normal colon**

The normal mucosal surface of the colon is composed by many crypts disposed as straight structures oriented to muscularis mucosae (Figs. 3, 4). The i.v. administration of fluorescein produces a strong staining of both surface epithelium and deeper layers of lamina propria. The images obtained by CLE show the luminal opening of the crypts as black holes surrounded by epithelial cells (columnar and goblet). In the colon, mucin containing goblet cells are readily identified as cells with dark inclusions (due to the fact that fluorescein is not highly miscible with mucins) [7]. The microvascularization is highlighted within the lamina propria in deeper parts of the mucosal layer (Fig. 5). The vasculature within the mucosa of the colon shows a typical honeycomb pattern that represents a network of capillaries circumscribing the mucosal glands [8]. Capillaries are brightly highlighted and different blood cells can be observed as dark inclusions in the lumen. Changes in vessel architecture can be used to diagnose inflammation and neoplasia [8]. In the colon, CLE may be useful to differentiate among normal tissue, non-neoplastic changes (such as inflammation and hyperplasia) and intraepithelial neoplastic changes during ongoing endoscopy.

**Colon cancer**

Colon cancer is the third most common form of cancer and the fourth most frequent cause of cancer deaths worldwide [10]. When it is symptomatic at detection, the prognosis is poor with an average five-year survival between 50 to 60% [11]. In order to improve the prognosis, colorectal
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cancer should be detected in earlier stages or even precursor stages. Early detection makes it possible to cure the patient by immediate endoscopic resection. Adenomatous polyps (Fig. 6), the most important risk factor for colon cancer, can be diagnosed with high accuracy taking into account the lack of epithelial surface maturation, crypt budding, altered vascular pattern, and loss of cell polarity [13].

Flat lesions are difficult to detect using standard endoscopic methods. New endoscopic imaging techniques such as autofluorescence imaging (AFI) and NBI have been investigated for the detection of suspicious areas in the gastrointestinal tract. However, the final diagnosis has to be confirmed by microscopic studies. Endomicroscopy was proposed as a feasible method to predict colon malignancy in vivo. The CLE aspects are quite typical: irregular epithelial layer with loss of crypts and goblet cells and major disorders in cells architecture (Figs. 7, 8). Furthermore, the vessels are usually dilated and distorted with increased leakage of contrast agents [8].

As a new hope for early detection of high-grade dysplasia, the combination of targeted peptide probes and visualization using CLE (“immunoendoscopy”) offers an appropriate method. A pilot human study in vivo showed that one of these peptides (VRPMPLQ) selectively binds to dysplastic tissue over normal mucosa. Imaging performed using a confocal microscope showed up to 50 times more fluorescence due to peptide binding to dysplastic polyps as compared with normal mucosa [12].

Ulcerative colitis

Patients with inflammatory bowel diseases, both ulcerative colitis (UC) and Crohn’s disease (CD) have a higher risk of developing colorectal cancer. It is considered that this risk is a result of persistent inflammation of the colon. Recent guidelines mention colonoscopy as a tool for screening this high-risk population. Random biopsies are recommended in order to detect dysplastic or neoplastic areas. However, multiple random biopsies are rarely performed. Colon examination using CLE can solve this
problem reducing significantly the number of biopsies per patient. It is not possible to examine the whole surface of the colon in the endomicroscopic mode, so the combination between endomicroscopy and chromoendoscopy seems to be an appropriate approach [7]. In vivo confirmation of neoplastic changes using CLE during colonoscopy may lead to major improvements in the clinical management of patients with UC. The inflammatory changes in UC are represented by crypt architecture alterations with normal cells inside [8]. The chronic inflammatory infiltrate produces thickening of lamina propria and the distance between crypts becomes larger. All these aspects are accompanied by increased vasculature of the mucosa (Figs. 9, 10). Confocal laser endomicroscopy can be used to confirm intraepithelial neoplasias in suspected areas, with a high degree of accuracy. Biopsies can thus be limited to targeted sampling of relevant lesions.

Conclusions

Endomicroscopy will play an important diagnostic role during gastrointestinal endoscopy in the future. The great advantage of CLE is that it avoids the diagnostic delay associated with conventional biopsy preparation and processing, enabling in vivo microscopic assessment of the tissue during real-time examination. Furthermore, CLE in association with targeted contrast-agents (immunoendoscopy) will selectively highlight dysplasia or early cancer. This will lead not only to real-time early diagnosis of neoplastic changes, but it will also modify the clinical management of these patients, with a possible decrease in the morbidity and mortality induced by colonic diseases.

Conflicts of interest

None to declare.
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References


