Endomicroscopy for Assessing Mucosal Healing in Patients with Ulcerative Colitis

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Abstract
The assessment of tissue healing has emerged as an important treatment goal in patients with inflammatory bowel disease. In patients with ulcerative colitis (UC), mucosal healing may represent the ultimate therapeutic goal due to the fact that the inflammation is limited to the mucosal layer. Mucosal and histological healing may indicate a subset of UC patients in long-term clinical, endoscopic and histological remission in whom immunomodulators, biologics, and even aminosalicylates may be withdrawn.

Confocal laser endomicroscopy allows the assessment of residual cellular inflammation, crypt and vessel architecture distortion during ongoing endoscopy, and therefore permits a real-time evaluation of histological healing in patients with ulcerative proctitis. Images of conventional optical microscopy and confocal laser endomicroscopy in patients with ulcerative proctitis in remission are presented.

Key words
Endomicroscopy – mucosal healing – ulcerative colitis.

Introduction
The assessment of tissue healing has emerged as an important treatment goal in patients with inflammatory bowel disease (IBD). It is associated with sustained clinical remission and reduces the rates of hospitalization and surgical resection, as well as the direct and indirect costs [1]. In patients with ulcerative colitis (UC), mucosal healing (MH) may represent the ultimate therapeutic goal due to the limited inflammation to the mucosal layer. Several endoscopic scoring systems have been developed using different items and definitions of MH. The easiest to use are the modified Baron score and the Mayo score [2, 3]. The endoscopic variables for the Baron score are friability, vascular pattern, bleeding, and ulcerations assessed by using a 5-points scale (from 0 to 4). Endoscopic variables of the Mayo score are erythema, vascular pattern, friability, bleeding, erosions and ulcerations, assessed with a 4-point scale (from 0 to 3); MH is defined by a score of 0 and 1. The weakness of these scores is the lack of discrimination between superficial and deep ulcerations. The Baron score, distinguishing three grades of activity, has been commonly used to evaluate the degree of endoscopic activity. Recently, the FDA recommended considering any friability as non-healed mucosa [4]. In 2007, the International Organization of IBD proposed a definition of MH in UC: the absence of friability, blood, erosions, and ulcers in all visualized segments of the gut mucosa [5]. Although total MH means, in theory, no visible lesions at all, the experts concluded that an abnormal vascular pattern, in the absence of the other features of the disease, is still compatible with MH [1]. Finally, MH may be used as ultimate therapeutic goal to select patients in whom stopping immunosuppressive or biologic therapy could be considered [1].

Moreover, there are experts who promote the concept of histological healing (HH) in patients with UC in remission and MH to characterize complete recovery of the colonic mucosa [1]. Histological healing means either absence of residual mucosal inflammation with distinctive changes of crypt architectural distortion and/or atrophy, or entirely normal mucosa [6]. Histological assessment of UC is inseparable from colonoscopic investigation and biopsy in current clinical practice. The assessment of inflammation activity by conventional colonoscopy is inaccurate in the prediction of acute inflammation in some cases, especially for those seeming to be in remission as evaluated by conventional colonoscopy. Histological assessment of inflammation in UC includes acute inflammatory cell infiltrates (polymorphonuclear cells in the lamina propria), crypt abscesses, mucin depletion, surface epithelial integrity, chronic inflammatory cell infiltrates (lympho-plasmocyte cells in the lamina propria) and crypt architectural irregularities.
Numerous studies have demonstrated that HH is possible with several medications [5, 8, 9]. In ulcerative proctitis, HH may indicate a subset of UC patients in long-term (1-2 years) clinical, endoscopic and histological remission in whom even aminosalicylates may be withdrawn [10, 11]. The use of confocal laser endomicroscopy (CLE) in the diagnosis of UC was reported recently by Kiesslich et al [12] and Watanabe et al [13]. The CLE assessment of inflammatory activity includes cellular infiltration, crypt architecture and vessel architecture [13]. Recently, a classification of inflammation activity in UC by CLE was proposed [14].

**Technical background**

The CLE developed by Pentax® and Optiscan® represents a regular endoscope which has integrated in the distal tip, a miniature confocal microscope. It allows the performance of CLE procedure during ongoing endoscopy, by placing the distal tip of the endoscope (the endomicroscope) in intimate contact with the mucosal surface. An argon ion blue LASER delivers an excitation beam wavelength of 488 nm with a maximum power output at the surface of the mucosa of less than 1 mW, allowing targeted endomicroscopic images to be captured. There are multiple images recorded at different depths of the mucosal layer, which range between 0-250 µm (z axis). The optical slices are parallel with the mucosal surface and have a 7 µm thickness, with a lateral resolution of 0.7 µm. The field of view is 475/475 µm [15]. The position of the focal plane (the depth of the captured image) can be controlled by two additional buttons placed on the control unit of the confocal endoscope - one button automatically resets the focal plane at the mucosal surface and the other can advance the focal plane towards deeper or more superficial layers, with a step of 4µm.

In order to obtain the CLE images, an exogenous fluorescence technique has to be used; there are several agents that can be used, some of them being administered systemically (fluorescein), others topically (acriflavin, tetracycline, cresyl violet) [16]. The most extensively used are acriflavine hydrochloride 0.05% in saline solution for topical application and fluorescein sodium 5-10 ml 10% solution for intravenous injection. Acriflavine stains only the superficial layers of the mucosa, including the cells nuclei, whereas fluorescein is distributed from the capillaries through the entire mucosa, showing the microvascular network and the connective tissue architecture [15].

**Clinical applications of CLE in the assessment of mucosal and histological healing**

Assessing systematically HH in UC patients in long-term (1-2 years) clinical and endoscopic remission, we can identify a subset of UC patients with complete remission, in whom chronic maintenance medication, even aminosalicylates, may be withdrawn. Considering that CLE allows the performance of assessing residual cellular inflammation, crypt and vessel architecture distortion during ongoing endoscopy, we have attempted to assess histological features by CLE in patients with ulcerative proctitis in clinical and endoscopic remission on 5-ASA for 2 years.

After being informed regarding the purpose of the examination, our patients were prepared for colonoscopy. Bowel preparation before CLE did not differ from that before conventional colonoscopy. The CLE device used was an EC3870K (Pentax, Tokyo, Japan). All patients were given a 5 ml intravenous injection of 10% fluorescein. The CLE procedure did not differ from those of conventional colonoscopy, with inflammation activity assessed by the Baron score. Only patients with Baron score 0 (normal mucosal appearance) were included in the CLE assessment. During the colonoscopy, the distal tip of the endoscope was placed gently on the observed mucosa with the endomicroscopy mode turned on. At least 2-3 Z-stacks of images were obtained and the endoscopist evaluated the crypt architecture, inflammatory cellular infiltrate and microvascular features. CLE images of observed mucosa were stored digitally on laser discs in a database which had been used before for CLE examination of the upper gastrointestinal mucosa and which allows further evaluation.
[17]. A targeted biopsy was performed for histological analysis. Biopsy specimens were fixed with 10% formalin and embedded in paraffin, and sections were stained with hematoxylin and eosin for histopathological examination. Two control groups consisting from patients with irritable bowel disease (control group A) (Fig. 1 A, 1B) and patients with active ulcerative proctitis (Baron score 3) (control group B) (Figs. 2A, 2B, 2C) underwent CLE and mucosal biopsies.

In patients with normal endoscopic appearance of the rectal mucosa (Baron 0) we observed two different patterns. A subset of patients presented a completely normal conventional microscopic and CLE appearance (Figs. 3A, 3B), while others showed distinctive and subtle changes in crypt architecture described as focal distortion of crypt architecture corresponding at CLE examination to an irregular arrangement of crypts and fluorescein leakage in the luminal opening of the crypts (Figs. 4A, 4B).

The therapeutic goals for IBD have changed dramatically over the past decade, from treatment of symptoms and control of the disease activity toward significant changes in the natural course of the disease, preservation of gut function and prevention of disabilities. In addition to MH, the incorporation of HH as an important end point of clinical trials and as a desirable individual goal in clinical practice in UC patients will consolidate the concept of sustained deep remission, and will allow reconsidering the natural history and long-term therapy in patients with UC. Therefore, if this goal will be achieved in prospective trials, the ability of CLE to assess HH will create a dedicated tool for evaluating the long-term outcome in patients with UC.
Conclusion and areas for future research

Confocal laser endomicroscopy is a reliable tool for real-time assessment of mucosal and histological healing in UC, allowing the assessment of inflammatory cellular infiltrate, crypt architecture and microvascular pattern. Further studies have to be aimed at assessing the role of CLE in the definition and evaluation of prognostic value of histological healing in UC.

Conflicts of interest

None to declare.

References