ASSESSMENT OF CRYPTITIS DEVELOPMENT IN ULCERATIVE COLITIS – ULTRASTRUCTURAL STUDY

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Summary

Ulcerative colitis is an inflammatory chronic disease primarily affecting the colonic mucosa. Neutrophils within epithelial structures, such as the crypt wall (cryptitis), or the crypt lumen and wall (crypt abscesses) or in association with crypt damage (crypt destruction) are helpful for the diagnosis.

To assess ultrastructural changes of the colonic epithelium in patients with active UC with special referral to the development of crypt abscesses. Methods: a total number of 28 patients, 20 with clinical manifestations of UC and 8 healthy subjects underwent colonoscopy. The biopsies obtained during colonoscopy, after specific preparation were stained using routine H&E histological methods using a light microscope Carl Zeiss Ergaval to establish the histopathological diagnosis.

The specimens for ultrastructural assessment were stained with uranyl acetate and lead citrate and studied with a JEM-1010 transmission electron microscope. We observed the rarefaction or even the absence of the microvilli, the reduction of goblet cells, the shatter of the epithelial junctions, cytoplasmic vacuolisation, dilatation of the endoplasmic reticulum, pycnotic nuclei, the destruction of the mitochondria and Golgi complexes which conducts in the end to the drastic reduction of the mucus production.

Our investigation has revealed that in acute stage of UC a loose cohesion of epithelial cells is present. We can say that, in the acute stage of ulcerative colitis, a great part of the ultrastructural changes are interrelated with the disorders of the epithelial cells. Cryptitis, crypt abscesses and crypt destructions are important in the development of the inflammatory process in UC.

**Key words:** ulcerative colitis, electron microscopy, crypt abscesses

Introduction

Ulcerative colitis (UC) was first described by the London physician Sir Samuel Wilkes in 1859 (Wilkes, 1859).

Ulcerative colitis is an inflammatory chronic disease primarily affecting the colonic mucosa; the extent and severity of colon involvement are variable. In its most limited form it may be restricted to the distal rectum, while in its most extended form the entire colon is involved.

Although the causes of IBD remain unclear, considerable progress has been made recently in the identification of important pathophysiologic mechanisms, and further and newer knowledge has been obtained from recent studies concerning their epidemiology, natural history, diagnosis and treatment (Ardizzone, 2003).

The microscopic pattern of UC is characterized by an inflammatory reaction with special distribution and structural abnormalities of the mucosa (Goldman, 1991).
Inflammation is characterized by increased intensity of the lamina propria cellular infiltrate with alterations of the composition and changes in distribution (Jenkins et al., 1997).

In UC, the infiltrate is more extensive and extends diffusely towards the deeper part (transmucosal). Accumulation of plasma cells near the mucosal base, in-between the crypt base and the muscularis mucosae (basal plasmacytosis), is common. Focal or diffuse basal plasmacytosis (combined with crypt distortion) is a strong predictor for the diagnosis of chronic idiopathic inflammatory bowel disease (IBD) and occurs in over 70% of the patients (Schumacher et al., 1994).

The presence of neutrophils, indicating a change in the composition of the inflammatory infiltrate, is another important feature. When combined with unequivocal epithelial cell damage, it indicates disease activity (Jenkins et al., 1997). This feature can be assessed with good reproducibility.

Neutrophils within epithelial structures, such as the crypt wall (cryptitis), or the crypt lumen and wall (crypt abscesses) or in association with crypt damage (crypt destruction) are helpful for the diagnosis, but the predictive value of these features is limited.

Cryptitis and crypt abscesses can indeed also be seen in infectious colitis, Crohn’s colitis and diversion colitis (Shepherd, 1991). In UC however, they are generally more common, being present in 41% of the cases. Crypt alterations are more common and more widespread; they are observed in 57–100% of cases.

Material and methods

We studied a total number of 20 patients, with clinical manifestations of UC or previously diagnosed with the disease and now being in relapse and 8 healthy subjects.

The patients were investigated in ambulatory or they were hospitalized for further investigation and treatment in Clinical County Hospital Oradea- 3rd Medical Clinic.

The examination was performed using an Olympus Exera CLE 145 videoendoscope. Colonic biopsies were taken during endoscopy from patients with UC and from healthy subjects.

The biopsies obtained during colonoscopy, after specific preparation were cut into 3-4 µ thick sections (8-10 per case). In order to establish the histopathological diagnosis and to include the case into a group of lesions, the first sections were stained using routine H&E histological methods using a light microscope Carl Zeiss Ergaval.

Later the specimens were fixed in 2.5 % phosphate buffered 0.1M glutaraldehyde sol. (pH 7.4) and then post fixed with 1% osmic acid in phosphate buffered (pH 7.4). After rinsing in phosphate buffer 0,15M, the pieces were dehydrated with acetone, embedded in Epon 812 and then obtained ultrathin sections (70nm), with the aid of ultramicrotome type Leica UC 6, stained with uranyl acetate and lead citrate. Afterwards sections were studied with a JEM-1010 transmission electron microscope (JEOL, Tokyo, Japan). Special attention was guided towards the development of crypt abscesses.

Results and discussions

From the histopathological point of view we observed that the active forms were characterized by the existence of a severe inflammatory infiltrate in the lamina propria with the predominance of neutrophils.

These aspects were confirmed by ultrastructural assessment as shown below (fig.1-2).
Ultrastructurally, we observed a marked accumulation of inflammatory cells right next to the crypts. The basal membrane was in some places disrupted or even absent and leucocytes were moving into the epithelial cells (fig.3). Also diapedesis of leucocytes throughout the basal membrane was encountered. Between the widened intercellular spaces of the epithelial cells there were erythrocytes and lymphocytes (fig.4). Mast cells were also met in some regions (fig.5).

Organelles of the epithelial cells surrounding the crypts were normal. Free ribosome and Golgi complexes were noted as well as some irregular. Goblet cells and widened intercellular spaces were seen in some of the specimens. The intercellular spaces are enlarged and the cellular components are altered.

Round dense mucous vesicles were also met along the apical surface. Microvilli presented marked edema and destructions.
So, in conclusion, we observed the rarefaction or even the absence of the microvilli, the reduction of goblet cells, the shatter of the epithelial junctions (Figure 6), cytoplasmic vacuolization, dilatation of the endoplasmic reticulum (Figure 7), pycnotic nuclei, the destruction of the mitochondria and Golgi complexes which conducts in the end to the drastic reduction of the mucus production (Figure 8 and 9).

Some goblet cells have an altered activity in Golgi complexes and they secrete insufficient amounts of immature granules.

Fig. 6 UC- active phase: the epithelial cell adhesion is profoundly altered and the tight junctions are destroyed

Fig. 7 UC- active phase: the vacuolization and lyses of the cytoplasm, endoplasmic reticulum dilatation

Fig. 8 UC- active phase: the microvillus border is destroyed, the rectal mucosal cells have pycnotic nucleus with irregular contour

Fig. 9 UC-active phase: the thickness of the mucosa is reduced, in mucosal cells the Golgi complex is flattened and the mitochondria has a rarefied matrix

The characteristic lesion in UC is represented by the existence of neutrophils in the glandular crypts (cryptitis and cryptic abscess) (Utsumi et al., 1999). The mucus depletion, edema and focal hemorrhage were alterations met during the active phases of the disease, things that were also remarked in other studies, like those made by Kruschewski et al. (1995) or McLaren et al. (2002). In active stage a loose cohesion of the epithelial cells is seen. The inflammatory cells and erythrocytes migrate from lamina propria towards the lumen passing through large, widened intercellular spaces.
Normally, only lymphocytes can be found among the colonic epithelial cells (Dobbins, 1986).

Ultrastructurally, there are important alterations of the cell structures in the active phase and in the quiescent phase. It is important to underline that in the affected area tissue necrosis might appear and it represents a precursory process of ulceration. In time, along with the destructive phenomenon, there is a healing/scarring and regeneration process (Nagy, 2007).

During the time special attention was paid to the pathologic composition of the mucus in ulcerative colitis.

The composition of the pathologic glycoproteins are similar to those in associated malignant tumors but in ulcerative colitis the process is reversible, coming back within normal limits after the acute inflammation diminishes. In our biopsy samples the goblet cells are drastically reduced in number. Along the apical surface there are small, dense “mucigenic” vesicles and this may be the cause for the pathologic composition of the mucous (Daniel, 2008).

Compared to the normal rectal mucosa – which has overloaded goblet cells, and where the release of the mucus is made according to the necessities into the lumen and the absorbent cells from around have a normal, thick microvilli – the affected mucosa from UC is displaying severe epithelial alterations.

Conclusions

Our investigation has revealed that in acute stage of UC a loose cohesion of epithelial cells is present. The inflammatory cells and erythrocytes migrating from lamina propria toward the lumen of the crypts could easily pass through widened intercellular spaces. So, we can say that, in the acute stage of ulcerative colitis, a great part of the ultrastructural changes are interrelated with the disorders of the epithelial cells. Cryptitis, crypt abscesses and crypt destructions are important in the development of the inflammatory process in UC.

References

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