HIGHER APOPTOSIS INDEX AND PROLIFERATION INDEX IN COLONOCYTES OF PATIENTS WITH ULCERATIVE COLITIS IN REMISSION

JAROSŁAW BUCZYŃSKI¹, Michał SPYCHALSKI¹, AGNIESZKA ŁAWSKA-WIERZCHNIEWSKA², ADAM DZIKI¹

Department of General and Colorectal Surgery, Medical University in Łódź¹
Kierownik: prof. dr hab. A. Dziki
Department of Pathomorphology, Medical University in Łódź²
Kierownik: prof. dr hab. W. Papierz

Ulcerative colitis (UC) is a non-specific inflammatory disease involving the mucosa and submucosa of the large bowel, ascending from the rectum. In extreme cases, defined as pancolitis, it may affect the entire large intestine.

The disease incidence is significantly higher in Caucasians living in developed countries, standing at 10 cases per 100000 persons per year. However, there is observed an alarming growth in the CU incidence. In a large-scale population study conducted in Sweden in the years 1965-1983, there has been found an increase in the incidence from 7 cases to 12 cases per 100000 persons per year (1). Unfortunately, Poland does not have accurately kept records of new UC cases, but there is seen a similar upward trend in the numbers of new cases as in the Western Europe.

The histological picture of acute phase of the disease comprises inflammatory infiltration of granulocytes, lymphocytes, plasma cells and histiocytes (2), involving the mucosa and submucosa. Plasma cells produce IgM, IgG and
IgA antibodies. Numerous lymphatic follicles are present in the inflammatory infiltrations, which leads to crypt relocation (3). Lymphatic follicles are present mainly in the rectum. Inflammatory infiltration causes epithelial damage and crypt abscess development. Atrophic and reparative processes lead to the irregularity of glandular ducts. Non-specific granulation is present on the abscess surface. The pseudopolyps developing commonly, visible on endoscopy, are fragments of congested inflamed mucosa. In remission, the mucosa is free of lesions macroscopically, but microscopy reveals changed glands with irregular ramifications.

The aetiopathogenesis of UC is multifactor. It is associated with genetic and immune disorders. Environmental factors have impact on disease manifestation as well. It is also suggested that infectious factors affect the development of this disease. The genetic background of this disease confirms its familial occurrence and much higher incidence in certain ethnic groups as compared with the general population.

To date, 12 chromosomal loci of genes potentially associated with this disease have been identified. They are located on chromosomes 1, 3, 5, 6, 12, 14, 16 and 19. The above genes include those coding for interleukin 1, interleukin 2B, interleukin 3 receptor, HLA, NKX2-3 and MST1 (4, 5). Yet another suspected genome region has been identified during research conducted by scientists from the University of Pittsburgh. It is a gene encoding ECM1 which strongly activates NF-κB, a protein being the key regulator of immune response. Its aberrations seem to be responsible for the increased risk of UC development.

The treatment of UC may be divided into the treatment in disease exacerbation and the treatment maintaining remission. Exacerbation treatment, i.e. an attempt at inducing remission, differs depending on the disease symptom intensity. The used drugs include glucocorticosteroids and immunosuppressants. The drugs used in remission, aimed at protecting the patient from disease recurrence, are 5’ASA derivatives.

Currently, the majority of experts agree that the 5’ASA derivatives should be used at the maximum well tolerated dose not producing side effects. In terms of maintenance therapy duration, it is believed it should be long. Treatment discontinuance may be attempted in specific cases only.

In the case of conservative treatment failure, i.e. steroid resistance or steroid dependence, or in the case of disease complications, surgical treatment is necessary, consisting in total proctocolectomy.

The large bowel mucosa is lined with simple columnar epithelium. It consists of intestinal crypts of approx. 300-400 µm in length. The crypt wall is made of large numbers of goblet, cylindrical and stem cells. Stem cells are located at the bottom of intestinal crypts and divide asymmetrically. Newly synthesised DNA is transferred to cells migrating towards the crypt apex. The apoptotic index in the healthy epithelium lining the large bowel is variable. At the crypt bottom, proliferation predominates. In the upper 2/3 of the crypt, the apoptotic index goes up. Depending on the measurement method, it ranges from 0.5% to 11%.

Apoptosis is clearly observed in the intestine of patients with UC (6). Apoptosis regulation disturbances, affecting its intensity and thus disturbing the cell-tissue homeostasis, are factors causing disease development. This is confirmed by other reports on the presence and intensity of apoptosis not only in epithelial cells, as in healthy individuals, but also in crypt cells at sites with pathological lesions (7).

The aim of the study was determination of the proliferation index and apoptotic index in colonocytes of patients with UC in remission, undergoing long-term treatment with 5’ASA preparations (mesalazine).

MATERIAL AND METHODS

The study involved the analysis of histopathological specimens of large bowel mucosa of patients with histopathologically confirmed UC, in clinical and endoscopic remission, receiving mesalazine preparations at a mean dose of 2 g daily as a remission maintenance treatment (8). The exclusion criterion was the use of steroids by the patient. All the patients meeting the inclusion criteria had a rectal biopsy specimen collected during check-up colonoscopy.

There were 23 patients qualified for the study, including 17 males and 6 females.
The mean patient age was 37. Patients were treated for UC at Klinika Chirurgii Ogólnej i Kolorektalnej (Department of General and Colorectal Surgery), Medical University of Lodz.

For the proliferation index determination, immunohistochemical staining with anti-Ki-67 monoclonal antibody was performed, according to the manufacturer instructions (Monoclonal Mouse Anti Human Ki-67 Antigen, Clone MIB-1, Cat. No M7240, Dako). Cells with stained nuclei were counted as positive. There was estimated the percentage of Ki-67-positive cells per 1000 colonocytes in regions of most intense staining (the so-called hot spots).

For apoptosis evaluation, immunohistochemical staining for Bax was performed, according to the manufacturer instructions (Polyclonal RabbitAnti-Human Bax, Cat. No A3533, Dako). Cytoplasmic reaction estimated by a semi-quantitative method was deemed as positive. During the assessment of the ratio of positive cells to all intestinal glandular cells in the examined specimen, the numerical scale was set, with 0 – <1% of positive cells, 1 – 2-25% of positive cells, 2 – 26-50% of positive cells, 3 – 51-75% of positive cells and 4 – 76-100% of positive cells.

RESULTS

The results are presented in figs. 1 and 2.

In the studied group, among 23 patients, the mean proliferation index, as determined by Ki-67, stood at 42.13% (maximum: 57%, minimum: 33%).

The mean Bax value, representing the number of apoptotic cells in the studied group, stood at 1.47 and fit in the 0-3 range. High Bax index, i.e. large numbers of apoptotic cells, was observed not only at the crypt bottom but also at the opening. Both the numbers of proliferating cells, calculated as the percentage of all cells, and the numbers of apoptotic cells, evaluated by a semi-quantitative method, were clearly higher in the studied material than the values observed for the healthy intestine.

DISCUSSION

To date, there have been several reports published, describing proliferation, apoptosis and regulatory factors in the healthy large bowel (6, 9). However the mechanisms of maintaining and functioning of this subtle yet resistant barrier between the environment full of bacteria, toxins and metabolites inside the intestine and the cells of the body are not fully known. The intestinal epithelium is renewed owing to proliferation at the crypt bottom and apoptosis at the apex. Cell migration along the crypts takes approx. 4-5 days. Apart from studies on the physiological phenomena of colonocyte apoptosis and proliferation, there have also been published findings on the proliferation and apoptosis mechanism disturbance in the intestine of patients with UC (10). Pathological intensification of apoptosis seems to lead to a disturbance in the cell barrier and initiation of an unfavourable sequence of events mediated by a number of compounds from the interleukin class and TNF.
In the present study, it was decided to search for apoptotic cells with the use of an immunohistochemical method employing antibodies against Bax – a potent activator of this process. This method, described in numerous publications, is reproducible and burdened with small error. The numbers of apoptotic cells were determined semi-quantitatively, with the use of an index, where: 0 – < meant apoptosis at the level of 1%, 1 – 2-25%, 2 – 26-50%, 3 – 51-75% and 4 – 76-100%.

Bax values clearly exceeded the normal range for healthy epithelium, which is 0.5-11%. The majority of our measurements in the semi-quantitative method ranged between 1 and 3, which corresponded to the percentage of apoptotic cells of 2-75%, which also significantly exceeded the normal range for healthy epithelium, and which is in agreement with other publications on the subject (6, 7, 10). Of note is the observed noticeable increase in the numbers of apoptotic cells at the crypt bottom and not at the opening, as in the healthy epithelium.

It should be emphasised that, when assaying cells expressing Bax, it was the cells of higher apoptotic readiness, i.e. proapoptotic cells, that were assayed. The Bax protein, belonging to the bcl-2 protein family is a proapoptotic protein, similarly to Bad, Bak and Bid, as opposed to bcl-2 and bcl-xl which protect the cells from apoptosis. Therefore, in further research, it would be beneficial to verify the obtained results by assaying the proteins involved in the apoptosis initiation phase (caspase-3 and caspase-9) and by staining the true apoptotic cells, e.g. by the TUNEL method.

The homeostasis between cell proliferation and apoptosis enables correct renewal of large bowel epithelium and ensures its proper functioning.

In inflammatory diseases and in neoplasms, the cell homeostasis is disturbed and proliferation is markedly intensified (11). In the studied patient group, the proliferation index – similarly to the apoptotic index – significantly exceeded the normal range, despite the absence of macroscopic features of inflammation.

Similar findings have been made previously in an animal model (dextran-induced intestinal mucositis in rats), where higher proliferation index (Ki-67) and apoptotic index were observed (12).

To date, few reports have been published on apoptosis in UC in remission. The majority of authors focus on examining the phenomenon in the acute phase of the disease (10). Therefore, the results of the present study should be treated as novel ones.

The present study is also associated with the chemopreventive effects of mesalazine preparations on the development of colorectal cancer (13). Large-scale studies have evidenced the chemopreventive activity of 5’ASA preparations towards colorectal cancer in the group of patients receiving such preparations due to UC. Although there are no unambiguous reports on the mechanism of its action, the majority of reports quote the chemopreventive action of mesalazine through apoptosis intensification (14, 15, 16).

The results of the present study undoubtedly confirm the marked intensification of cell death through apoptosis in the epithelium of patients with UC.

It would also be worthwhile to further analyse the changes in expression of genes regulating apoptosis and the intensified proliferation in UC in terms of intensified dysplasia in crypt cells and increased tendency to neoplastic transformation.

**SUMMARY**

The results of the present study confirm the markedly accelerated renewal of large bowel epithelium in patients with UC. Of note is the fact that the studied group included patients in clinical and endoscopic remission, and such studies have not been reported to date. This leads to the conclusion that the clinical and endoscopic picture may be misleading and inaccurate. The genetic and immune disturbances in UC occur despite the treatment. Mesalazine, by affecting only certain pathways associated with UC, maintains remission (17), yet without providing “molecular” remission. Therefore, it appears that the results of the present study provide yet further evidence on the need for caution in discontinuing the maintenance treatment.

The present study also fits in the discussion on the chemopreventive activity of 5’ASA and its metabolic pathways in which the regulation
of cell death undoubtedly plays a crucial role.

In addition, the present study should certainly be treated as a pilot one. It would be beneficial to reproduce the study on a larger material, to include the evaluation of the studied parameters at different stages of the disease and – potentially – their examination in cases of attempted discontinuation of the maintenance treatment, as well as to assess the potential correlation between their values and the risk of disease exacerbation.

REFERENCES