Short report

Expression of markers of apoptosis and proliferation in patients with nonspecific ulcerative colitis

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Abstract: The analysis of the expression of markers of apoptosis and proliferation in 80 patients with ulcerative colitis has been carried out. The reference group consisted of 15 practically healthy people. In biopsy materials of the colonic mucosa the expression of immunohistochemical markers Ki-67 and p53 was determined. The reduction of the proliferative activity of epithelial cells of the colon and increase in the activation of apoptosis with the growing severity of ulcerative colitis, the increase of the colon lesion range and the disease endoscopic activity has been revealed.

Keywords: nonspecific ulcerative colitis, markers, apoptosis, proliferation.

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Introduction

Ulcerative colitis (UC) is one of the most severe diseases of the gastrointestinal tract, leading to disability and death of patients [1].

In the recent decades in different countries an increased incidence of ulcerative colitis was registered, which is largely due to improved diagnosis of this pathologic process.

Currently, in the diagnostics of UC the comprehensive approach with the use of radiological, endoscopic, histological research methods is employed. One of the promising ways to assess the state of the mucous membrane of the colon is immunohistochemistry with the definition of markers of proliferation and apoptosis [1].

Among the methods of immunohistochemical investigation most widely are used the markers of apoptosis bcl-2, Bax and p53 [2]. It should be noted that the p53 protein is detected in many transformed cells. Its functions are aimed at the prevention of damaged genetic information transfer from one generation of cells to another, due to apoptosis initiation as well. The high content of p53 leads to increased concentration of Bax in the cell and to the decrease in the concentration of bcl-2, which promotes cell death due to apoptosis [2].

Several antigens are used as proliferation markers. Proliferating cell nuclear antigen (PCNA) is involved not only in cell proliferation, but also in DNA reparation after injury [3], which makes this antigen conditionally specific for the cellular cycle, since DNA repair can be carried out in the resting phase [1]. Another antigen, significantly associated with the phases of the cell cycle, is Ki-67. Expression of this protein occurs during presynthetic phase, increases during the cell cycle and decreases sharply in the phase of mitosis [4]. This protein, in contrast to PCNA, is not involved in DNA repair [5]. Expression of Ki-67 makes it possible to identify the cells in all phases of the cell cycle except the resting phase [6, 7].

The purpose of the study was to analyze the expression of markers of apoptosis and proliferation in patients with ulcerative colitis, depending on the duration and severity of the disease, localization and activity of the inflammatory process.

Materials and methods

The study group comprised of 80 patients with ulcerative colitis at the age of 18 to 66 years (33 women and 47 men). The reference group comprised of 15 healthy subjects. Examination of patients was performed with the use of clinical, laboratory, endoscopic and morphological methods, as well as by immunohistochemical study of biopsy specimens of the colonic mucosa.

Patients with ulcerative colitis were divided into groups depending on the severity of clinical symptoms, disease duration, localization of the inflammatory process and its endoscopic activity.

Proliferative activity of the cells was determined by the proliferative index of Ki-67 as follows:

PI (Proliferation index) Ki-67% = X (number of nuclei immunopositive for Ki-67) × 100 / X_1 (the total number of nuclei),

where *X* - the number of nuclei in the field of view of the microscope. The counting was carried out in at least 10 fields of view.

Apoptosis was assessed by the expression of p53 protein in the surface and glandular epithelium of the colon.

After the statistical processing of the data and tests for equality of variances and distribution normality it has been proved that the sampling does not meet the law of normal distribution, that is why the non-parametric tests were used for the groups comparison. For description of the quantitative features we used the median, upper and lower quartiles – Me (Q_1, Q_2).

Results

In healthy individuals *PI* Ki-67 was 54 (46, 67), indicating a high proliferative activity of the cells of the colon (*Table* 1).

As shown in *Table* 1, the increase of *PI* Ki-67 in patients with disease duration of 1 year to 5 years, compared with group 1 (p=0.06), can be attributed to increased cell proliferation, reflecting the repair processes in the colonic mucosa against a background of the therapy. With increasing duration of disease the cells' ability to proliferate decreases, evidence of which is the low *PI* Ki-67 in the third group of patients.

Depending on the severity of clinical manifestations of ulcerative colitis a change of *PI* Ki-67 was also revealed (*Table* 1). *PI* Ki-67 decreased in patients with the moderate form of disease (p=0.0006) and the severe form (p=0.002), compared with patients having mild course, there was also a difference between healthy individuals and patients with ulcerative colitis of any degree of severity (p=0.04).

The analysis of *PI* Ki-67 dependence on the localization of the inflammatory process revealed a significant decrease in expression of the marker in patients with ulcerative colitis who had the total colonic involvement (pancolitis): 30 (28, 31), whereas in case of distal colitis *PI* was 45 (37, 46) (p=0.0009).

It is also obvious from *Table* 1, that an increase in disease activity is leading to *PI* Ki-67 decrease. There are significant differences between patients with I and II (p=0.002), I and III (p=0.02) degrees of activity.

The expression of p53 was not observed in any case in the epithelium of the colonic mucosa of practically healthy people.

The results of the expression of this protein in patients with ulcerative colitis are presented in *Table* 2.

Expression of p53 marker depended on the disease severity (12.5% - mild, 35.7% - moderate and 50%- severe). Statistically significant differences were obtained between the groups of healthy individuals and patients with ulcerative colitis who had moderate and severe stages of disease (p=0.03).

In patients with distal colitis the positive result was obtained only in 22.2% of cases, with left colitis - in 18.1% of patients. In case of pancolitis the expression of p53 was revealed in 100% of cases (p=0.03 compared to distal colitis). It should be noted that the expression was most intense in areas with signs of epithelial metaplasia.

Besides, the dependence on endoscopic disease activity has been discovered: the expression of p53 (p=0.04) increased with its raising.

Table 1. Proliferation index (*PI*) Ki-67 of epithelial cells of the colonic mucosa of healthy subjects and patients with ulcerative colitis

Groups	n	*PI* Ki-67, %	P-level
Reference group	15	54(46;67)	
Duration of disease			
<1 year	21	32 (28, 37)	p_0 =0.0002
1-5 years	33	39 (31, 45)	p_0 =0.0005, p_1 =0.06
>5 years	26	35 (22, 47)	p_0 =0.0003, p_1 =0.05
Severity			
mild	21	45 (37, 47)	p_0 =0.01
medium	47	34 (30, 40)	p_0 =0.00001, p_2 =0.0006
severe	12	30 (28, 31)	p_0 =0.002, p_2 =0.002
Localization of lesions			
distal	26	45 (37, 46)	p_0 =0.007
sinistral	39	35 (31, 40)	p_0 =0.00003, p_3 =0.01
total	15	30 (28, 31)	p_0 =0.0002, p_3 =0.0009
Endoscopic activity			
I	28	44 (36, 46)	p_0 =0.004
II	35	32 (28, 40)	p_0 =0.00001, p_4 =0.002
III	17	34 (31, 35)	p_0 =0.002, p_4 =0.02

p_0 – p-level for difference from the control group, p_1 - p-level for difference with disease duration of less than 1 year, p_2 - p-level for difference with mild disease course, p_3 - p-level for difference with distal localization of disease, p_4 - p-level for difference with the 1st degree of endoscopic activity.

Table 2.Expression of p53 protein in patients with ulcerative colitis

	Duration of disease					
	Up to 1 year		*From 1 to 5 years*		*More than 5 years*	
	n	*%*	*n*	*%*	*n*	*%*
Positive	6	33.3	2	12.5	6	42.8
Negative	12	66.7	14	87.5	8	57.2
	Severity of disease					
	Mild		*Medium*		*Severe*	
	n	*%*	*n*	*%*	*n*	*%*
Positive	2	12.5	10	35.7	2	50.0
Negative	14	87.5	18	64.3	2	50.0
	Process localization					
	Distal		*Sinistral*		*Total*	
	n	*%*	*n*	*%*	*n*	*%*
Positive	4	22.2	4	18.1	8	100
Negative	14	77.8	18	81.9	0	0
	Endoscopic activity					
	I		*II*		*III*	
	n	*%*	*n*	*%*	*n*	*%*
Positive	4	20	6	27.2	4	66.7
Negative	16	80	16	72.8	2	33.3

Discussion

The analysis of obtained results showed that in case of ulcerative colitis the proliferative activity of the epithelial cells of the mucous membrane of the colon greatly reduces and the expression of p53 protein increases compared to the mucosa without inflammatory changes, which is consistent with the literature data [8, 9].

It should be noted that in cases with the disease duration up to 1 year, the lowest *PI* Ki-67 is observed, which may be due to the absence of pathogenetic therapy.

However, we have not found such information in the available literature.

Conclusion

With an increase of disease course severity, volume of colon involvement and endoscopic activity in patients with ulcerative colitis there is a decrease in proliferative activity of the epithelial cells of the colon and increase in the activation of apoptosis.

Conflict of interests

The study was conducted in the framework of main research of the Saratov State Medical University n.a. V.I. Razumovsky (Saratov, Russia).

Reference

1. Kalinina AV, Loginova AF, Hasanova AI. Gastroenterology and hepatology: diagnostics and treatment: doctor`s handbook. Moskow, Russia: MEDpress-inform, 2011. 864 p.
2. Aruin LI, Kapuller LL, Isakov VA. Morphological diagnostics of gastrointestinal diseases. Moskow, Russia: Triada-H, 1998. 496 p.
3. Brown DC, Gatter KC. Monoclonal antibody Ki-67: its use in histopathology. *Histopathology* 1990; 17(6): 489-503 (PMID: 2076881).
4. Bruno S, Darynkiewich Z. Cell cycle dependent expression and stability of the nuclear protein detected by Ki-67 antibody in HL-60 cells. *Cell Prolif* 1992; 25: 31-40 (PMID: 1540682).
5. McCormick D, Chong H, Hobbs C, Datta C, Hall PA. Detection of the Ki-67 antigen in fixed and wax- embedded section with the monoclonal antibody MIB-1. *Histopathology* 1993; 22: 355-360 (PMID: 8514278).
6. Bromley M, Rew D, Becciolini A, Balzi M, Chadwick C, Hewitt D, Li YQ, Potten CS. A comparison of proliferation markers (BrdUrd, Ki-67, PCNA) determined at each cell position in the crypts of normal human colonic mucosa. *Eur J Histochem* 1996; 40(2): 89-100 (PMID: 8839702).
7. Holt PR, Moss SF, Kapetanakis AM, Petrotos A, Wang S. Is Ki-67 a better proliferative marker in the colon than proliferating cell nuclear antigen? *Cancer Epidimiol Biomarkers Prev* 1997; 6(2): 131-135 (PMID: 9037564).
8. Ivashkin VT, Lapina TL, Bondarenko OYu, et al. Processes of apoptosis and proliferation in the pathology of gastrointestinal tract and liver. *Russian Journal of Gastroenterology, Hepatology, Coloproctology* 2002; (6): 38-43 [Article in Russian].
9. Osadchuk AM, Osadchuk MA. Morphofunctional regeneration of epithelial cells in the colon and apudocytes in the pathogenesis and prognosis of the course of non-specific ulcerous colitis. Clinical Medicine 2006; 84(12): 35-39 [Article in Russian] (PMID: 17294881).

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