Parameters of Apoptosis and Proliferative Activity in the Stomach in Patients with Rheumatological Diseases

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ABSTRACT

Programmed cell death (PCD) is one of the key links in the pathogenesis of gastritis and peptic ulcer disease. In the course of the study, the proliferative-apoptotic status of epithelial cells of the gastric mucosa was determined in rheumatological patients at the onset of the disease and during treatment with NSAIDs in comparison with peptic ulcer, which allows us to conclude that reparative processes are reduced.

Keywords: apoptosis; proliferation; epithelial cells of the gastric mucosa.

Tens of thousands of scientific papers are devoted to the mechanisms of apoptosis development. Currently, a huge amount of material has been accumulated, indicating the role of proliferation processes and programmed cell death in maintaining the structural integrity of the gastric mucosa. Structural homeostasis (homeomorphosis) of tissues is provided by a balance between new cell formation (mitosis) and their death (apoptosis). Apoptosis plays a huge role in the homeostatic control of the dynamic balance between cell proliferation and their elimination, its launch and maintenance is controlled by the corresponding anti- and pro-apoptotic factors, among which p53, Bcl-2 and PUMA play the main regulatory role [3,4]. The predominance of mitoses leads to hyperplasia, the predominance of apoptosis leads to atrophy [1].

It is known that gastroduodenal complications often occur in rheumatological patients [5], which significantly affect the course and results of treatment of rheumatological patients [7]. Perhaps this is due to the negative effect of drugs used to treat this category of patients. The study of the state of cell renewal in the stomach and in rheumatological patients is also important from the position that a wide arsenal of drugs used for the treatment (NSAIDs, corticosteroids, etc.) of rheumatological patients are potentially dangerous due to the development of gastroduodenal side effects.

Aim: comparative analysis of the relative content of epithelial cells subjected to necrosis, the values of mitotic and apoptotic indices in the gastric mucosa in an equivalent number of patients with rheumatological diseases at the onset of the disease and during treatment with NSAIDs and duodenal ulcer.

Materials and methods. The paper presents the results of a comprehensive screening examination of 40 patients with rheumatological diseases (RD), including 20 patients at the onset of the disease, 20 patients with a history of 1 to 10 years or more. The control group consisted of 20 patients with duodenal ulcer (DUD). All three groups of patients were comparable in terms of gender and age.

Determination of markers of proliferation and apoptosis was carried out by immunohistochemical method. To determine apoptosis and proliferation, immunohistochemical research methods were used. For this purpose, biopsy material was used in the amount of 60 samples taken from the antrum of the stomach (in 40 patients with rheumatological diseases and 20 in patients with duodenal ulcer). Processing of the biomaterial was performed according to standard histological methods with filling in paraffin.

To determine the level of expression of the p53 protein, p53 (clone DO-7) Dakopatts (Denmark) was used as the primary monoclonal antibody (mAb), and PCNA mAb (clone PC-10) Dakopatts (Denmark) was used to assess PCNA activity. The results of determining the expression of the p53 protein and PCNA were evaluated by the percentage of positively reacting cells per 500 analyzed. The apoptosis index (AI) was defined as the number of stained bodies divided by 1000 cells and multiplied by 100%, and the mitotic index (MI) was calculated as the number of stained nuclei divided by 500 cells and multiplied by 100%.

The obtained data were subjected to statistical processing on a Pentium I computer using the Statgraphics software package (USA). Significance of differences (P) was calculated using Student's t-test. Differences were considered significant at $P<0.05$. The studies were carried out in the laboratory of genetics of the RCRC of the Ministry of Health of the Republic of Uzbekistan.

Research results and discussion. In this work, we studied the death and proliferation of cells of the gastric mucosa in rheumatological patients at the onset of the disease and during treatment with NSAIDs.

As can be seen from the data presented in Table 1, the mitotic index (MI) in rheumatological patients was 0.6±0.1%, in patients with PU this indicator was 3.5 times higher. The index of apoptosis in patients with PU was also higher than in rheumatological patients. x patients by 1.6 times. Consequently, in rheumatological patients in the gastric mucosa, epithelial cells undergo apoptosis to a lesser extent than in patients with PU. A similar picture can be traced in relation to proliferation. Considering the fact that in normal cells of the stomach, the apoptosis index (AI) is about 3% [1], in rheumatological patients, the death of epithelial cells in this way is higher (1.6 times) than in healthy people, which indicates the stimulation of apoptosis in RD. .

Table 1

Some indicators of cell renewal in the gastric mucosa in rheumatological patients

Patient groups	Показатели				
	МИ (Митотический индекс п=6000в %)	АИ (Апаптотический индекс в п=5000%)	Некроз % п=3000	Коэффициенты	
				АИ/МИ	АИ/некроз
Peptic ulcer of duodenum (control)	2,1± 0,2	8,0± 0,5	2,4± 0,3	3,8	3,3
Rheumatologists	0,6± 0,1	4,9± 0,4	1,6± 0,2	8,2	3,1
*P	<0.05	<0.05	<0.05≤P<0.1		

Note: *P<0.05 vs. control.

The study of the ratio of apoptosis and mitosis (proliferation) of epitheliocytes in the examined groups of patients shows that if the value of this ratio in rheumatological patients is 8.2, then in patients with PU it is 3.8, respectively. Consequently, the studied coefficient is 2.2 times higher in rheumatological patients.

Given that under physiological conditions, a balance must be maintained between the process of proliferation and cell death, it becomes obvious that under conditions of both rheumatological diseases and peptic ulcer disease, this ratio is violated, due to the predominant activation of cell death processes. This, of course, can lead to a cellular deficiency of the gastric mucosa and is fraught with the risk of developing atrophic gastritis [9]. The results obtained show that the imbalance between renewal and death of epitheliocytes is more pronounced in rheumatological patients than in PU. This, apparently, is due to the fact that under conditions of increased cell death of the gastric mucosa by apoptosis, there is a more pronounced stimulation of proliferative processes, and new, young epitheliocytes are formed from the germinal zone. This is possible if there is a sufficient compensatory possibility of the growth zone of the mucous membrane. And in the conditions of rheumatological patients, due to the potential systemic lesion of the connective tissue structures of the stomach, the growth zone may initially suffer and hence the relatively low intensity of proliferative processes.

It is known that there are two ways of cell death: apoptosis and necrosis [8]. The path of death "chosen" by the damaged cell depends on many factors, including the cell type, its energy status, the nature and degree of damage, the state of the body's immune system, etc. [10].

Considering that under the conditions of the studied pathologies, especially with duodenal ulcer, there are a number of factors that damage the epithelium of the gastric mucosa, leading to cell death by the type of necrosis. We separately analyzed the characteristics of necrobiotic processes of the gastric mucosa in the examined groups of patients.

As can be seen from the data presented in Table 1, in rheumatological patients, the proportion of cells undergoing necrosis is 1.6 ± 0.2%, and in patients with PU this figure is 1.5 times higher.

Consequently, under conditions of both rheumatological diseases and ulcerative colitis, cells in the gastric mucosa also die by necrosis, and this is relatively pronounced in patients with ulcerative disease. Considering that necrosis, unlike apoptosis, is only a manifestation of the pathological process, it becomes clear that under conditions of rheumatological diseases, although the primary attack locus is “far” from the gastric mucosa, damage to the epithelial lining of this organ occurs.

Interesting results were obtained when analyzing the ratio of AI/necrosis, despite the presence of clear differences between the compared groups, both in terms of necrosis and apoptosis, the ratio of AI/necrosis was almost the same in both groups of patients. This indicates that regardless of the number of cells subject to death, the ratio of both pathways of cell death remains unchanged. This is fully consistent with the opinion of C.J Zeiss [380], about the existence of the so-called "apoptotic-necrosis continuum", determined by the cross-interactions between the reactions of cell death and the preservation of homeostasis.

It is known that the process of programmed cell death is regulated in a complex manner through humoral factors, glucocorticoids, and cytokines; there is also gene regulation through special regulatory proteins [11, 12]. Among the special proteins that regulate programmed death, the p53 protein plays a central role [6]. This protein regulates the expression of genes involved in cell cycle blockade, or interacts with proteins involved in programmed cell death.

In this regard, to assess the readiness of cells for apoptosis in RB, the expression of p53 protein in gastric epithelial cells was studied. The results of these studies are presented in Table 2.

Table 2

Expression of the p53 protein in the gastric mucosa in rheumatological patients.

Группы больных	Кол-во	Положительная экспрессия белка p53

	образцов	Кол-во положительных наблюдений	Частота p53 положительных клеток, в % n=500
Ревматологические болезни:			
в дебюте	20	1	14,00+1,55
на фоне НПВП	20	13	61,60+2,2 *
Язвенная болезнь ДПК (контроль)	20	10	16,30+1,65

Note: *P<0.05 vs. control.

As can be seen in RB at the onset of the disease, a positive reaction to the p53 protein is observed only in one case out of 20 observations, which is 5%. At the same time, in the stomach of patients with duodenal ulcer, a positive reaction to the p53 protein is observed in 50% of cases. In RBs with a certain "experience", against the background of ongoing therapy with anti-inflammatory drugs, this figure reaches up to 65%. Therefore, in RP with a sufficient history and receiving symptomatic therapy, in the gastric mucosa cells with a positive reaction to the presence of the p53 protein are significantly more common.

The study of the expression level of the p53 protein in gastric epithelial cells shows (Table 2) that in RB at the onset of the disease, the expression level of this proapoptotic protein is practically comparable to that in patients with PU. However, as the duration of the disease increases and anti-inflammatory drugs are taken, the level of p53 protein expression increases by 4.4 times compared with the onset of the disease. Therefore, in RP in the gastric mucosa there is a high level of expression of the pro-apoptotic protein p53. Even in the conditions of the "debut" of the disease, the expression of this protein in the cells of the gastric mucosa in rheumatological patients is comparable to PU. With the duration of the disease and the use of NSAIDs, the number of cells with degraded DNA and prepared for programmed death increases, since it is the p53 protein that has the ability to prevent the fixation of genetic damage and initiate apoptosis in cells with DNA defects.

It is known that, despite the similarity of some destructive processes during apoptosis and necrosis, the fundamental difference between apoptosis is the presence of genetic control and specific protein regulators of the apoptotic process [8]. Therefore, apoptotic cell death is also called programmed cell death, which is designed to maintain the constancy of the number and cull defective cells. Apoptosis in a healthy organism is balanced by its physiological regeneration. At the same time, the process of regeneration, like apoptosis, is also programmed and has genetic control. One of the markers of this control is the expression of the PCNA protein.

We also studied the expression of the PCNA protein in the cells of the gastric mucosa of patients with RB. As can be seen from Table 3, under the conditions of the pathology under study, both at the onset and during NSAID treatment, all samples of the gastric mucosa showed a positive reaction to the presence of the PCNA protein. A similar picture can be traced in samples obtained from patients with duodenal ulcer.

Table 3

Expression of the PCNA protein in the gastric mucosa in rheumatological patients.

Группы больных	Кол-во образцов	Положительная экспрессия белка PCNA	
		Кол-во положительных наблюдений	Частота PCNA положительных клеток, в % n=500

Ревматологические болезни: в дебюте	20	20	95,20± 0,96*
на фоне НПВП	20	20	78,5±1,8
Язвенная болезнь ДПК (контроль)	20	20	82,1±1,7

Note: *P<0.05 vs. Control

Analysis of the expression level of PCNA protein in gastric epitheliocytes shows that if the value of the studied indicator in the debut of rheumatological diseases is 95.2 ± 0.96%, then in patients with the "experience" of the disease, against the background of NSAID therapy, it decreases by 18%.

Therefore, as the disease “chronizes”, the expression level of the PCNA protein, a marker of regeneration or proliferation in gastric epitheliocytes, decreases, and the gastric mucosa becomes less capable of cell renewal than at the onset of the disease. As evidenced by the increase in the AI / MI coefficient in RB, it is much higher than unity, i.e. there is an imbalance between the death of epitheliocytes with their regeneration. It should also be noted that if the level of expression of the PCNA protein in the cells of the gastric mucosa at the onset of rheumatological diseases is 16% higher than that in peptic ulcer, then in RB with "experience" on the background of NSAID therapy, this indicator is almost comparable to the results in peptic ulcer. diseases (Table 3). At the same time, the mitotic index in RB, as our results show (Table 1), is 3.5 times lower than in patients with PU.

Thus, the results of the conducted studies indicate that in RP in the gastric mucosa there is an activation of apoptosis of epithelial cells, which becomes more pronounced against the background of anti-inflammatory therapy. At the same time, the balance between the process of cell death and the process of regeneration in the gastric mucosa is disturbed due to the predominance of apoptosis over mitosis. In general, disturbances in the processes of cell renewal in the gastric mucosa in RD at the onset of the disease are comparable to those occurring in patients with PU. And in conditions of rheumatological diseases with a relatively long course (against the background of the use of NSAIDs), disturbances in the processes of cell renewal in the gastric mucosa become more pronounced than in patients with PU.

Therefore, from the conducted morphological studies of the gastrointestinal tract in RB, it should be noted that, in principle, the identified disorders, as well as clinical and functional signs, are similar to those disorders that occur in patients with diseases of the gastrointestinal tract, in particular the HD zone. At the same time, only quantitative differences are noted depending on the duration of the course of the disease. However, here it is necessary to pay attention to the genesis of these structural disorders. It can be assumed that in patients with RD at the onset, unlike gastrointestinal diseases, the locus of primary lesions is located not in the parenchyma (epithelial) cell, but in the stroma, which is inhibited as RD progresses, affecting the growth zone, exhausting its proliferative potential, has an indirect effect on parenchymal cells. This, apparently, is why in the initial stages of the development of RD from the gastrointestinal tract, changes in the mucosa are predominantly dystrophic in nature, without visible destruction. And as the history lengthens, these changes deepen, and the drugs used have a direct cytodestructive effect on the “bare” epithelial cells of the gastrointestinal tract. Consequently, in RB with a long course, the parenchyma (epithelial layer) of the gastrointestinal tract is subjected to an “attack” from two sides: from the connective tissue stroma (continued antigenic attack) and from the outside, from the cavity of the digestive tube (aggressive effects of drugs - NSAIDs). This is also confirmed by our morphological studies of

various parts of the gastrointestinal tract in this category of patients. From the above, it becomes clear that not only the development of morphological disorders that occur in conditions of rheumatological pathology and pathology of the gastrointestinal tract of an independent nature, but also the need to use as part of the correction of secondary disorders of the gastrointestinal tract in RB as a means of reducing the antigenic attack on the connective tissue stroma of the gastrointestinal tract, as and agents that have a cytoprotective effect on the epithelial cells of this tract.

It is known that an important indicator that determines the ability of tissues to recover after damage is the parameters characterizing the intensity of cell renewal processes: the degree of death and proliferation, as well as their ratio (B.K. Nurgalieva, G.A. Khamidulina, V.T. Ivashkin et al., 2005). If the process of cell death prevails over the process of proliferation, then cell "collapse" occurs - atrophy, and if, on the contrary, hyperplasia occurs (A.V. Kononov, S.I. Mozgovoy, M.A. Livzan et al. 2005.) . In assessing the processes of cell death, it is important to determine the type of cell death: apoptosis or necrosis. Although both types of cell death ultimately lead to a decrease in the number of functioning cells, however, the death pathways chosen by cells are ambiguous from the standpoint of cell renewal processes, in particular proliferation. Necrosis is the result of an unplanned event and occurs spontaneously; apoptosis is formed as a clearly regulated, genetically determined process [2].

If the issue of cellular renewal from the standpoint of apoptosis and prolifer The nature of the violations of these processes in the gastrointestinal tract in the conditions of rheumatological pathology remains unresolved, especially with regard to the dependence on the duration of the course of RD and the intake of drugs, in particular NSAIDs.

In connection with the above, in this work, we studied the indicators of cell renewal (apoptosis, necrosis, proliferation, etc.), as well as genetic markers (p53, PCNA) of this process in the stomach in examined patients with RD (at the onset and under long-term conditions). course of pathology) in a comparative aspect with patients with PU.

The results of studies in this direction indicate that in RB in the gastric mucosa, apoptosis of epitheliocytes is activated, which becomes more pronounced against the background of anti-inflammatory therapy (Fig. 1). Along with this, the balance between the processes of cell death and repair of cells of the gastric mucosa is disturbed due to the predominance of apoptosis over mitosis.

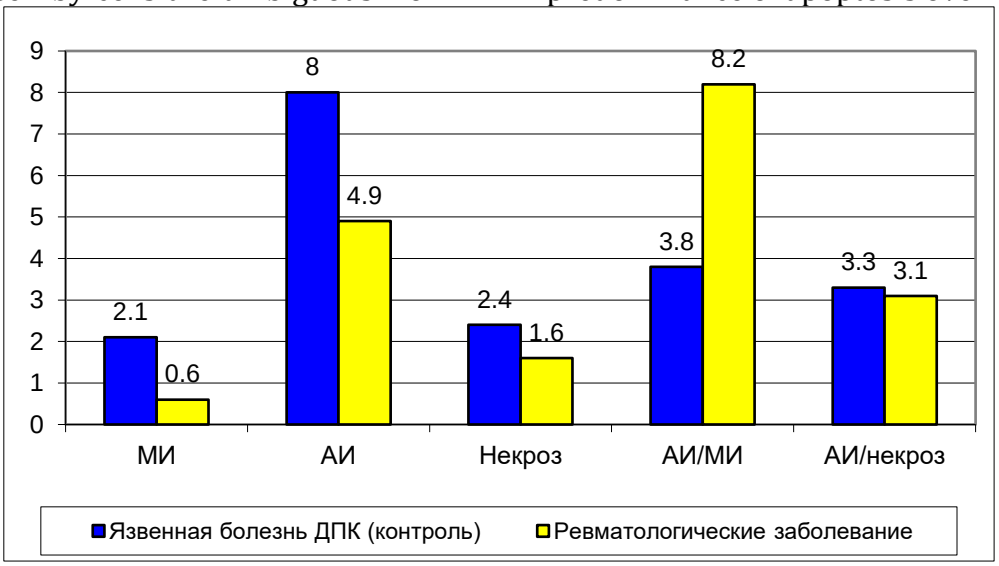

Fig. 1. Some indicators of cell renewal in the gastric mucosa in rheumatological patients

In general, disturbances in the processes of cell renewal in the gastric mucosa in RB are comparable to PU, as evidenced by shifts in the expression of genetic markers P-53 and PCNA (Fig. 2,3). With a long course of RD and the use of NSAIDs, disturbances in the processes of cell renewal in the gastric mucosa become more pronounced than in patients with PU.

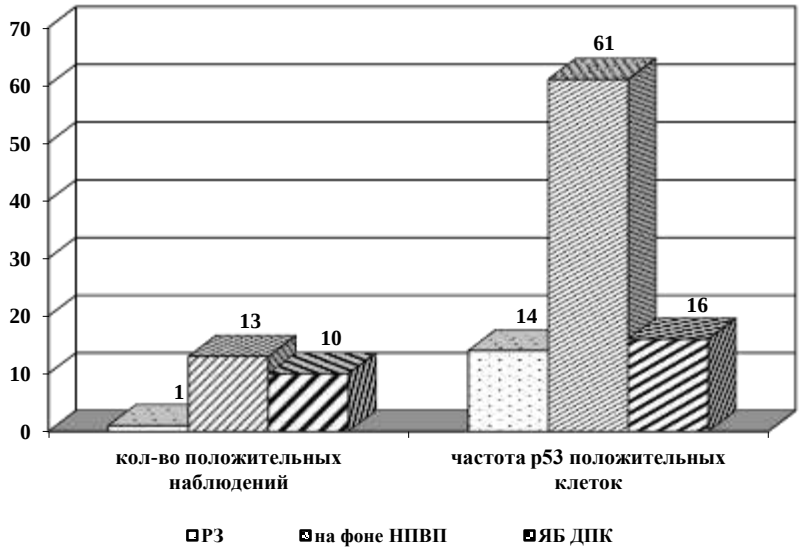

Fig.2. Expression of p53 protein in gastric mucosa in rheumatological patients

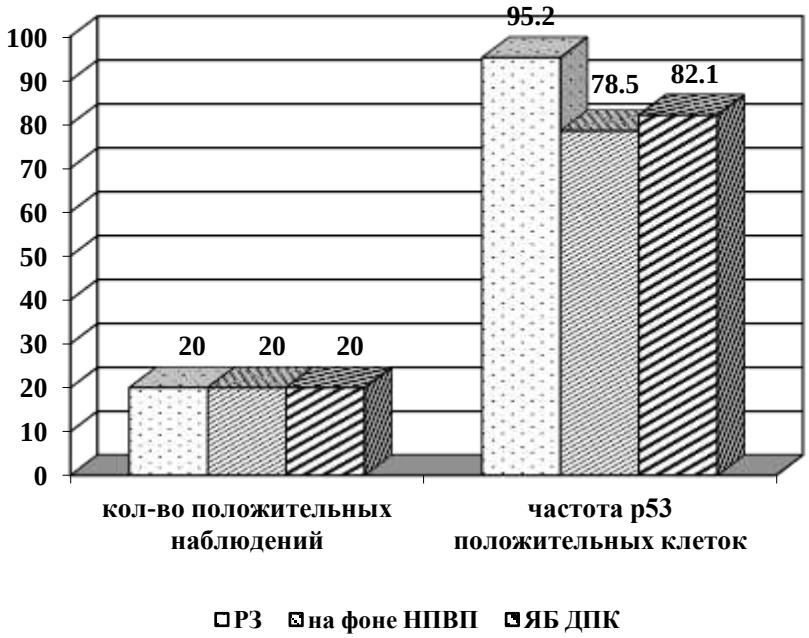

Fig.3. Expression of PCNA protein in gastric mucosa in rheumatological patients

Conclusion. Thus, the results of the conducted studies allow us to conclude that RB has a gastrointestinal lesion, even before the use of drugs (NSAIDs), and from all parts of the gastrointestinal tract. The lesion of the gastrointestinal tract in RB is confirmed by clinical and functional, endoscopic, microbiological, structural and biochemical research methods. The severity of the manifestation of gastrointestinal lesions in RB depends on the duration of the disease. If at the onset of the disease, clinical symptoms prevail over other signs, then with an increase in the duration of the history of the disease, the clinical symptoms, on the contrary, are inferior to other manifestations of the gastrointestinal tract.

In RB, there is a marked increase in the processes of cell death due to the predominance of necrotic death over apoptotic and a sharp decrease in the proliferative activity of the gastric epithelium, which is confirmed by genetic markers of apoptosis and proliferation. These patterns are less pronounced than in gastric ulcers, especially at the onset of the disease.

Thus, the primary damage to the connective tissue structures of the gastrointestinal tract, on the one hand, the

cytodestructive effect of drugs used to treat RD, on the other hand, has a destabilizing effect on the parenchymal cells of the digestive tube, creating conditions for the occurrence of functional (in the debut) and organic changes in this system. These changes undoubtedly have a negative impact on the course of the underlying disease, aggravating its clinical manifestations and reducing the effectiveness of the treatment.

Thus, the conducted studies indicate that RB has clinical, biochemical, functional, microbiological disorders of the gastrointestinal tract. These changes are observed not only in patients with a certain duration of the course of RD, but also in the onset of the disease. In addition, they are not equally represented depending on the gastrointestinal tract and the duration of the disease. Used for the treatment of rheumatological pathology, drugs, in particular NSAIDs, contribute to the aggravation of existing disorders of the digestive tube.

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