Artrofoon as Alternative Preparation in the Treatment of Uncomplicated Forms of Nonspecific Ulcerative Colitis

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Therapy of uncomplicated nonspecific ulcerative colitis with artrofoon effectively reduces the duration and number of relapses. During remissions, the count of apudocytes, serotonin-, melatonin-, vasointestinal peptide-producing, and mast cells and the parameters of cell homeostasis against the background of artrofoon therapy were much closer to the normal than during treatment with salofalk.

Key Words: *artrofoon; proliferating cell nuclear antigen; cyclin D_1; apoptosis; diffuse endocrine system; nonspecific ulcerative colitis*

Nonspecific ulcerative colitis (NUC) is a severe chronic disease characterized by diffuse inflammatory process localized on the surface of the colon mucosa (CM). Etiological treatment of this condition is not yet developed, while pathogenetic therapy is far from ideal, because of high incidence of local and general side effects caused by the corresponding drugs, relapses, and difficulties in attaining remissions. On the other hand, the development of NUC is associated with progressive disturbances in functional morphology of colon apudocytes and cell renewal in the colon, which creates conditions for epithelial dysplasia and colon cancer development [3,4]. The search for new effective and safe preparations for pathogenetic therapy of NUC is an actual problem.

Artrofoon, a preparation containing ultralow doses of affinity purified antibodies to TNF-α, is promising in this respect. It showed good results in the treatment of rheumatoid and psoriatic arthritis. However, the efficiency and safety of this preparation in the treatment of inflammatory colon diseases are to be verified.

Here we compared therapeutic efficiency and safety of artrofoon *vs*. salazopreparations in patients with uncomplicated course of NUC.

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MATERIALS AND METHODS

A total of 80 patients with uncomplicated NUC not requiring administration of glucocorticoids were examined. In all patients, the volume of injury was limited by distal colitis. Patient's age varied from 18 to 67 years (mean 39.5±2.2 years). The patients were divided into two equal groups. In group 1, salofalk in low doses (2 g per day) was administered *per os*. Group 2 patients received artrofoon (2 sublingual tablets 4 times a day). Dynamic observation was performed for 6 months; the patients received maintaining therapy with the above-specified preparations in constant doses. Examination of all patients was carried out according to the same program including clinical, endoscopic, and laboratory methods, morphological and immunomorphological studies of CM with evaluation of apoptosis index and measurement of proliferating cell nuclear antigen (PCNA) and cyclin D_1. The reference group included patients with irritable bowel syndrome.

For detection of apoptotic nuclei, the samples were impregnated after Moser. Apoptotic cell death was evaluated by the index of apoptosis (%) as the ratio of the number of apoptotic nuclei stained after Moser to the total number of nuclei (×100). PCNA was detected

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using monoclonal antibodies (clone PC10, Sigma, titer 1:1000). Cyclin D_1 content was assayed using monoclonal antibodies (Novocastra, titer 1:500).

Proliferative activity of cells was evaluated by proliferation indexes (in %): the ratio of the number of PCNA- or cyclin D_1-immunopositive nuclei to the total number of nuclei per 1 mm^2 section area (×100). The indexes were estimated for 10 visual fields in 3 sections of the studied biopsy material. The test area for evaluation of proliferation indexes included not less than 2000 cell nuclei.

Immunohistochemical method was used for verification of apudocytes. Commercial antibodies to chromogranin A (Dako, 1:50), serotonin (Dianova, 1:100), melatonin (CIDtech Res. Inc., 1:200), and vasointestinal peptide (VIP; Dako, 1:100) were used as primary antibodies.

RESULTS

In patients with NUC, the total number of endocrine cells reacting for chromogranin A increased and considerably surpassed the corresponding parameters in healthy individuals and patients with irritable bowel syndrome. The counts and functional activity of apudocytes producing serotonin and melatonin (EC_1 and EC_2 cells, respectively) increased in NUC compared to the corresponding parameters in patients with irritable bowel syndrome. The content and functional activity of mast cells and apudocytes producing VIP (D_1 cells) considerably decreased in NUC. Electron microscopy revealed hyperplasia of secretory granules and activation of endoplasmic reticulum in EC cells and functional exhausting of VIP-producing and mast cells.

It can be assumed that enhanced production of melatonin in NUC directly stimulates cell proliferation, while increased content of melatonin-producing cells in CM plays a compensatory role. Enhanced production of melatonin explains surface character of the inflammatory process in CM during NUC and relatively low percent of bowel wall perforations in this pathology [1].

Melatonin exhibits pronounced antioxidant properties [5]. It directly regulates colon peristalsis by

TABLE 1. Number of Endocrine and Mast Cells (per 1 mm^2 CM) and Parameters of Cell Renewal of Colon Cells in Patients with Uncomplicated Course of NUC during Relapses and Remissions (*M*±*m*)

Parameter	Irritable bowel syndrome (*n*=20)	NUC	
		group 1 (*n*=40)	group 2 (*n*=40)
Total population of apudocytes	15.17±0.52	24.95±0.48* 17.93±0.35^{+}	24.31±0.63* 16.35±0.34^{+o}
EC_1 cells	5.59±0.18	8.66±0.12* 6.43±0.22^{+}	8.53±0.25* 5.98±0.15^{+o}
EC_2 cells	3.58±0.22	11.14±0.49* 8.01±0.45^{+}	10.96±0.54* 6.25±0.37^{+o}
D_1-cells	4.13±0.32*	1.89±0.09* 2.82±0.12^{+}	1.97±0.12* 3.22±0.14^{+o}
Mast cells	7.09±0.20	3.96±0.16* 5.82±0.12^{+}	3.85±0.14* 6.27±0.17^{+o}
Index of PCNA, %	57.16±2.14	28.6±1.6* 37.11±0.84^{+}	28.0±1.3* 42.40±0.63^{+o}
Index of cyclin D_1, %	35.47±1.74 35.93±1.56	16.99±0.45 17.79±0.20^{+}	15.87±0.75* 20.23±0.88^{+o}
Index of apoptosis, %	2.46±0.22 2.32±0.18	5.20±0.25* 4.10±0.16^{+}	5.52±0.20* 3.69±0.19^{+o}

Note. Numerator: parameters during relapse, denominator: parameter during remission. $p<0.05$ compared to: *patients with irritable bowel syndrome, $^{+}$parameters during relapse, ^{o}group 1.

stimulating (in high doses) or inhibiting (low doses) smooth muscles of the gastrointestinal tract. It can be hypothesized that high concentration of melatonin in the colon led to the development of diarrhea.

Increased production of serotonin by apudocytes in CM is a pathological process leading to intestinal dyskinesia and tenesmus [6]. Serotonin hyperproduction stimulates blood clotting, induces spasm of microvessels, and leads to colon wall edema, which is inevitably associated with CM ischemia and necrosis with the formation of erosions, ulcers, and bleeding. It is known that serotonin stimulates noceceptove receptors and its hyperproduction is probably responsible for pain syndrome in NUC [7].

In group 1 patients, remission of NUC was attained after 36.4±3.2 days and in group 2 patients after 34.2±2.1 days. During further 6-month observation, relapses occurred in 11 patients of group 1 (27.5%) and in only 3 patients of group 2 (7.5%).

During clinical and endoscopic remission of NUC, the number of apudocytes, melatonin- and serotonin-producing cells decreases, but their number still considerably surpassed the normal. The population of mast cells and VIP-producing cells remained low. During remission, the functional morphological parameters of apudocytes in group 2 patients were closer to normal than in group 1. This determined lower frequency of inflammatory process relapses in group 2 patients. These disturbances can be explained by residual functional and structural changes in the large intestine in NUC. Increased content of serotonin-producing cells explains chronic pains in patients with NUC, colon dyskinesia during remission, and possible microcirculatory disturbances in the large intestine. Peristalsis disturbances in patients with NUC are determined by changes in VIP production. Decreased number of mast cells in inactive stage of the disease has a negative impact on blood circulation in the intestinal wall due to microthrombus formation against the background of decreased production of heparin by these cells. Chronic ischemia of the mucosa is a potent inductor of colon cell apoptosis and fibrogenesis. NUC relapses are accompanied by considerable inhibition of proliferative activity and activation of colon cell apoptosis. (Figs. 1-3).

In group 2 patients, parameters of cell renewal during remission more markedly tended to normal than in group 1 patients. No side effects were noted during artrofoon treatment. In the group receiving salofalk, skin itch requiring administration of H_1-histamine receptor blockers was observed in 2 patients (5%).

Thus, the development of NUC is associated with hyperplasia and hyperfunction of the total population of apudocytes and enterochromaffin cells producing melatonin and serotonin, against the background of

Fig. 1. Irritable bowel syndrome. Immunohistochemical reaction for PCNA, ×120.

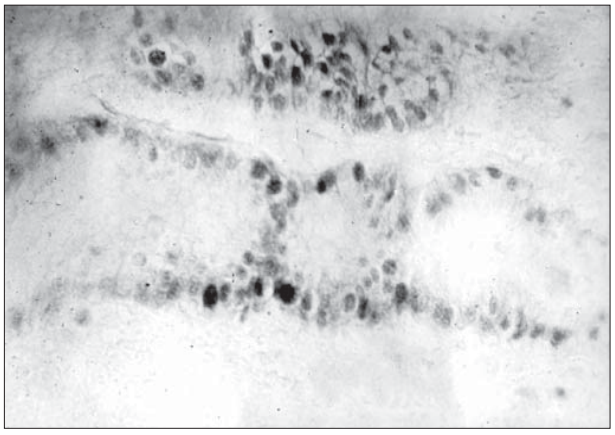

Fig. 2. NUC. Immunohistochemical reaction for PCNA, ×120.

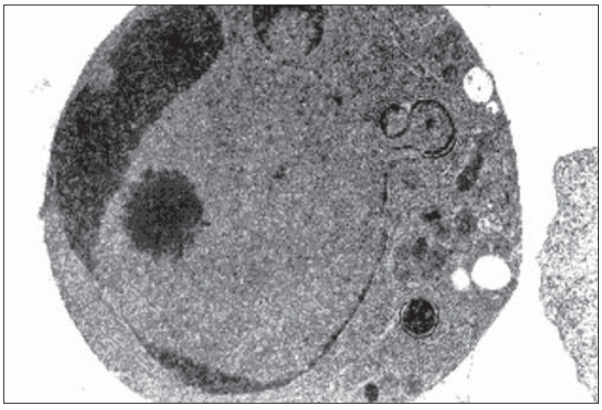

Fig. 3. NUC. Marginalization of chromatin in apoptotic nucleus, ×8000.

reduced population and functional exhaustion of VIP-producing and mast cells. Preserved functional morphology of apudocytes in the large intestine during NUC remission provides stability of residual clinical symptoms.

Disturbed functional morphology of apudocytes in the large intestine determines considerable decrease in proliferation parameters and activation of apoptosis of epithelial cells in CM.

During clinical and endoscopic remission of NUC, indexes of apoptosis and proliferation markers (PCNA and cyclin D_1) considerably deviate from the corresponding parameters in healthy individuals and patients with irritable bowel syndrome; this maintains cell homeostasis disturbances and leads to progression of morphological changes in CM.

The use of artrofoon maintains NUC remission in a greater number of patients compared to therapy with low doses of salofalk. The parameters of functional morphology of apudocytes and markers of cell homeostasis during NUC remission in patients receiving artrofoon were closer to normal values than in patients receiving low doses of salofalk.

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