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Treatment of Experimental Ulcerative Colitis

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The effects of infliximab, an anticytokine drug, on the course of inflammatory process was studied on the model of ulcerative colitis induced by injection of picrylsulfonic acid. Infliximab prevented the development of toxic dilatation and a drop of bioelectric activity of smooth muscles via maintenance of activity of the intramural nervous system neurons.

Key Words: *experimental ulcerative colitis; infliximab*

Inflammatory diseases of the intestine (IDI), including ulcerative colitis (UC) and Crohn's disease (CD), remain one of the most serious unsolved problems of modern gastroenterology. The incidence of IDI is significantly lower than the incidence of other diseases of the organs of digestion, but by severity, incidence of complications, and mortality they rank among the first in the structure of gastrointestinal diseases [4].

Certain progress has been attained in the treatment of IDI, but the efficiency of glucocorticoid therapy remains low in an appreciable part of patients because of progressive relapsing course and development of grave life-threatening complications.

The results of Mayo Clinic studies indicate that prolonged response to steroid hormone therapy is attained in just 49% patients receiving this treatment for the first time, while 22% patients develop steroid dependence and 29% are resistant to this treatment and colectomy has to be resorted to [6,7]. A total of 20-40% patients are in need of serious surgical interventions, after which the disease often relapses. These problems necessitate the development of new therapeutic trends for IDI patients, *e. g.* anticytokine therapy. Available data indicate that monoclonal antibodies, proinflammatory cytokine inhibitors, rapidly arrest relapses, provide a lasting remission, and reduce the need in hormones in many patients with hormone-resistant and hormone-dependent CD and UC. One of these drugs is infliximab. However, up to 1/3 of patients do not respond to anticytokine therapy (primary inefficiency of infliximab) [8]; patients regularly receiving maintenance infusions of infliximab later develop secondary inefficiency [7].

We studied the effects of infliximab on the motor evacuatory function and morphology of experimental UC.

MATERIALS AND METHODS

Experiments were carried out on Wistar rats (n=24; 250-270 g) under Nembutal narcosis (40-60 mg/kg). Ulcerative colitis was induced by injection of 45-50% ethanol solution of picrylsulfonic acid into the cecal lumen. The hypo- and hyperkinetic status of the cecum, ileum, and ascending colon during UC creation and on day 10 of its development were evaluated by the level of electromotor activity (EMA) with bipolar surface electrodes with contact surface of 1.5-2.0 mm^2. Registration was carried out on a multi-channel Nichon-Konden polygraph.

The role of anticytokine therapy was studied in a series of experiments on 6 rats, injected with infliximab (30 mg/kg) 1-4 min before UC induction.

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The morphology of the cecum was studied on day 10 of experiment. Tissue specimens were fixed in 10% formalin, dehydrated in ascending alcohols, embedded in paraffin, and stained with hematoxylin and eosin. Changes in the cecal wall were examined under a microscope at ×240-600.

The data were statistically processed by Statistica 6.0 software using Student's *t* test. The differences were considered significant at $p<0.05$.

RESULTS

The basal cecal EMA was characterized by slow-wave activity at a frequency of 12.5±0.8/min and amplitude of 0.08±0.01 mV. Three or four low-amplitude waves were recorded in the low-frequency slow wave band in 25% cases; high-amplitude (0.13±0.02 mV) high-frequency (0.60±0.08/min/100 slow waves) spike activity was recorded in 90% measurements. Basal cecal EMA could be characterized as rather intense.

The basal ileac EMA was characterized by high-frequency (20.1±1.8/min) and medium-amplitude (0.11±0.02 mV slow waves. Spike activity was recorded in 37.5% cases, the frequency of spikes was 0.34±0.05/100 slow waves, and amplitude was 0.12±0.04 mV. Hence, the basal motor activity of the ileum was rather intense, as spike activity was recorded in many cases.

Basal EMA of the ascending colon was characterized by medium-frequency (10.9±0.8/min) and medium-amplitude (0.13±0.02 mV) slow waves. Spike activity was observed in half of the cases: the incidence of spikes was 0.30±0.06/100 slow waves, amplitude 0.06±0.02 mV. Hence, the basal motor activity of the ascending colon was rather intense with significant spike activity.

Injection of picrylsulfonic acid into the proximal cecal lumen was associated with reduction of the ileac motor activity: the frequency of slow waves reduced to 16.8±0.6/min (17.5%; $p<0.05$) at stable amplitude. Spike activity pattern changed: the incidence of spikes was 0.65±0.05/100 slow waves and amplitude was 0.05±0.01 mV. Hence, the motor activity of the ileac decreased in UC with subsequent gradual reduction during the next 10 days (Table 1).

Injection of picrylsulfonic acid into the proximal cecal lumen was associated with reduction of the cecal motor activity: the frequency of slow waves decreased to 8.7±0.5/min (30.1%; $p<0.05$), the amplitude also reduced (to 0.05±0.01 mV, 37.5%; $p<0.05$). No spike activity was detected, this indicating a significant reduction of the smooth muscle bioelectric activity. Further reduction of slow-wave activity of the proximal cecum was observed after 10 days of UC.

Injection of picrylsulfonic acid into the proximal cecal lumen led to reduction of motor activity of the ascending colon: slow wave frequency reduced to 9.9±1.2/min (9.1%; $p<0.05$), amplitude decreased to 0.09±0.01 mV (30.7%; $p<0.05$). Spike activity was detected in 73% cases (frequency 0.45±0.02, 30%; $p<0.05$; amplitude 0.05±0.01 mV, 16.6%; $p<0.05$). These data indicated a reduction of the smooth muscle slow-wave activity and emergence of spontaneous spike activity. Further slight reduction of slow-wave activity of the ascending colon was observed after 10 days of UC simulation.

Chronic UC was induced after pre-injection of infliximab. The animals remained active throughout the next 10-12 days, they exhibited normal appetites and manifest exploratory activity.

Creation of UC after pre-injection of infliximab led to development of two opposite reactions of the cecal EMA: inhibition, with the slow-wave frequency reducing to 6.7±0.8/min (23%) and amplitude reaching 0.04±0.01 mV (20%; $p<0.05$) and increase of spike activity (spike frequency 2.3±0.4, amplitude 0.03±0.01 mV; Table 1). Hence, smooth muscle basal bioelectric rhythm reduced in experimental UC induced after pre-injection of infliximab, while the contractile activity of these muscles increased. Presumably, this latter fact prevented the development of toxic dilatation of the colon, observed in UC patients.

Ileac EMA in experimental UC under conditions of infliximab treatment was characterized by stable frequency of the slow-wave activity (16.6±1.2) and an increase of its amplitude (to 0.14±0.02 mV, 28.2%;

TABLE 1. Cecal Slow-Wave EMA in UC under Different Conditions

Gastrointestinal compartment	Ulcerative colitis		Ulcerative colitis under conditions of infliximab treatment	
	frequency/min	amplitude, mV	frequency/min	amplitude, mV
Ileum	16.8±0.8	0.11±0.02	16.6±1.2	0.14±0.02
Cecum	8.7±0.5	0.05±0.01	6.7±0.8	0.04±0.01
Ascending colon	9.9±1.2	0.09±0.01	7.8±0.7	0.09±0.01

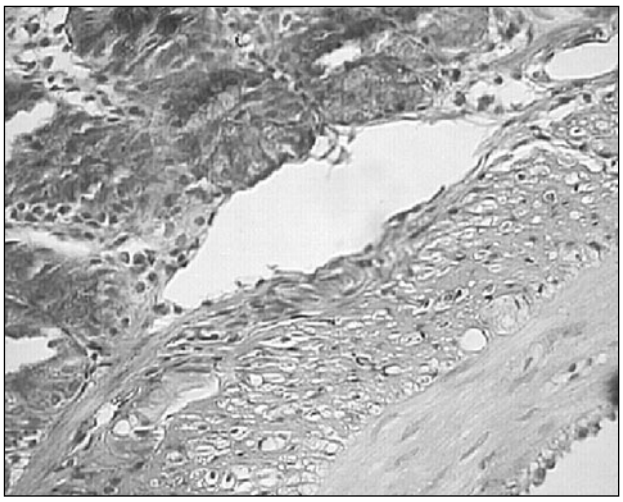

Fig. 1. The rat cecum in experimental UC: submucous edema, crypt abscesses (hematoxylin and eosin staining, ×600).

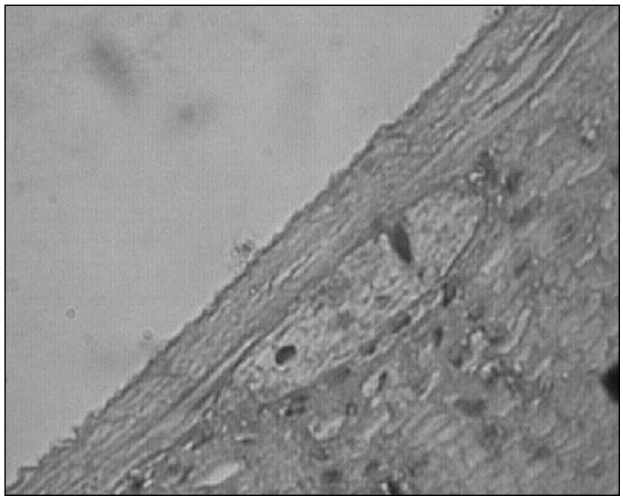

Fig. 2. The rat cecum in experimental UC: vacuolar degeneration of intermuscular plexus ganglion neurons (hematoxylin and eosin staining, ×600).

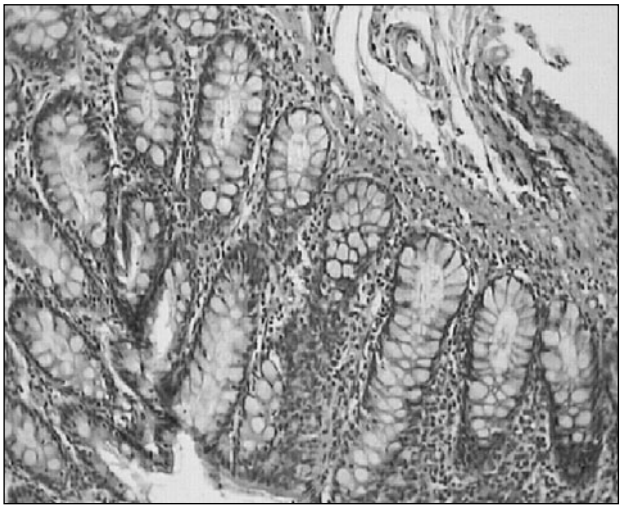

Fig. 3. The rat cecum in experimental UC induced after infliximab pre-injection (hematoxylin and eosin staining, ×250).

$p<0.05$). Spike activity was detected in 75% cases and was characterized by increase of the frequency to 0.84±0.08/100 slow waves (29.2%; $p<0.05$) and of amplitude to 0.07±0.01 mV (40%; $p<0.05$). Hence, anticytokine therapy to a certain measure blocked the development of ascending inflammatory reaction.

The ascending colon EMA in UC created under conditions of infliximab treatment was characterized by low frequency of slow-wave activity (7.8±0.7; 21.2%; $p<0.05$) at a stable amplitude. Spike activity was 2-fold more rare and was characterized by frequency increase to 0.70±0.05/100 slow waves (55.5%; $p<0.05$) and a reduction of the amplitude (0.04±0.01 mV, 20%; $p<0.05$). Hence, anticytokine therapy inhibited the development of descending inflammatory reaction.

Studies of the cecal wall morphology showed unevenly edematous cecal mucosa on days 10-12 of UC (Fig. 1), infiltrated with plasma cells and lymphocytes. Sites of epithelial cell desquamation and destruction were seen in the surface epithelium. Dilatation of blood vessels and hemorrhages were found. The subepithelial region was presented by granulation tissue infiltrated with various cells, including polymorphonuclear leukocytes and lymphocytes. Foci of polymorphonuclear leukocytes were found near some crypts, penetrating into the epithelium and crypt lumen. Cryptic abscesses were unfolding. Blood capillaries contained erythrocytes. Development of neuronal vacuolar degeneration was found in the intermuscular nervous plexus ganglia (Fig. 2).

The cecal mucosa in UC created after infliximab injection was characterized by well-developed granulation tissue; numerous lymphocytes and fibroblasts were seen. Macrophages and plasma cells were rare. Goblet cell hyperplasia, crypt cleavage, and numerous goblet cells emerging among the surface epithelial cells were found (Fig. 3). No vessels with destroyed walls or vacuolar degeneration of the intermuscular nervous plexus neurons were detected.

The morphology of the colonic mucosa indicated a high proliferative activity and reparative potential of infliximab.

Experimental UC was associated with high mortality. Pre-injection of infliximab before UC creation reduced the animal mortality 2-fold.

Our results indicated that the development of UC led to primary stimulation of EMA and its subsequent reduction in the cecum, ileum, and ascending colon. Anticytokine therapy inhibited the development of ascending and descending inflammatory reaction. Anticytokine therapy effect on the basal bioelectric rhythm was presumably explained by improvement of the function of the smooth muscle intramural pacemakers. The course of UC was less malignant after pre-injection of infliximab; the motoricity of the stu-

died compartments of the small intestinal and colorectal compartments little changed due to prevention of vacuolar degeneration of the intramural nerve plexus neurons and inhibition of the pronounced cytotoxic effect of T-lymphocytes.

TNF-α is the key cytokine in the cytokine cascade. It does not circulate in the blood in health. In enteric inflammations, it works as an active pro-inflammatory agent. This cytokine stimulates the induction of other pro-inflammatory cytokines (IL-1 and IL-6) and leukocyte migration by increasing endothelial permeability, expression of adhesion molecules by endothelial cells and leukocytes and plays the key role in the development of organ disorders of various kinds in human inflammatory diseases [2,3,5]. Use of monoclonal antibodies to TNF-α is one of the trends of anticytokine therapy for IDI. Infliximab is the first drug of this kind; it has been used in Russia for the treatment of rheumatoid arthritis, ankylosing spondilitis, psoriasis, CD and UC since 2001 [1].

Infliximab prevents the development of the so-called intestinal dilatation in IDI, a condition prognostically unfavorable in experiment and in clinical setting.

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