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Research Article

Pharmacological correction of ulcerative colitis with dalargin

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Abstract

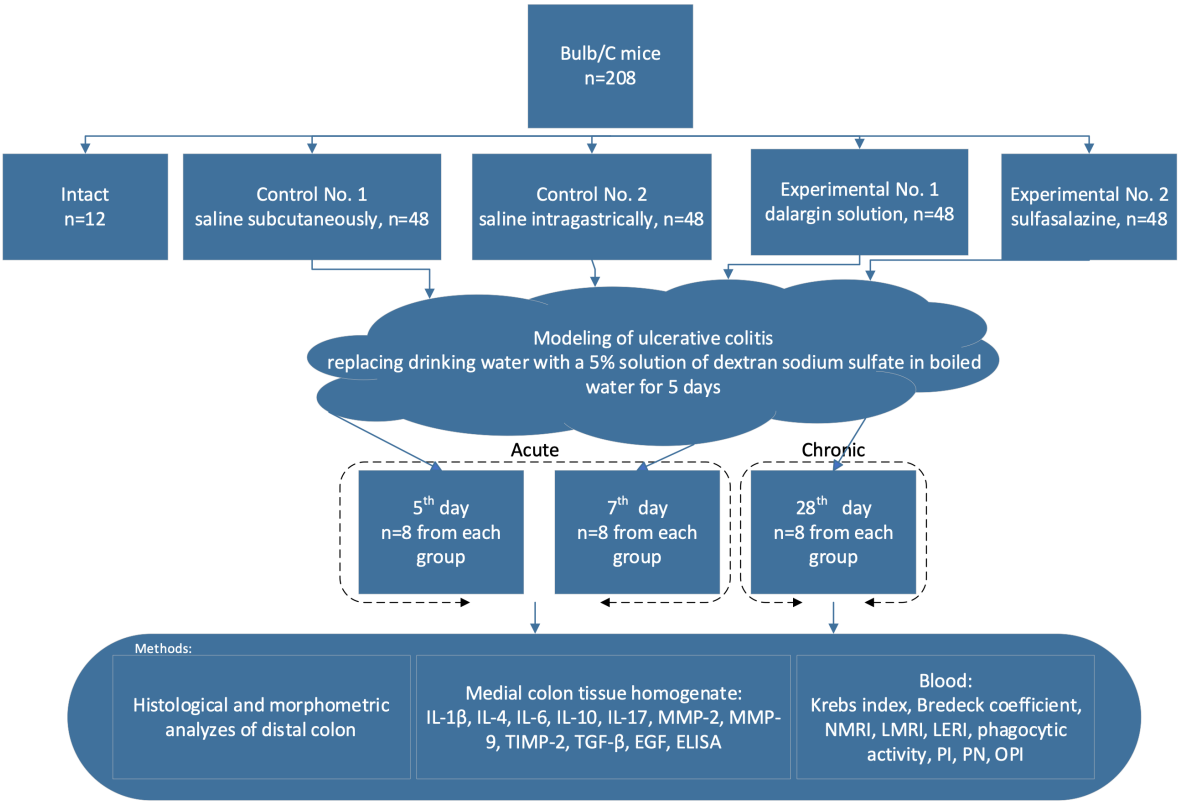
Introduction: Ulcerative colitis is a chronic colonic disease with frequent relapses, affecting mainly people of active age. The effectiveness of existing treatment methods remains low. Dalargin has the following pharmacological effects: antioxidant, membrane-stabilizing and immunomodulatory, which are important in treating ulcerative colitis. **The aim of the study** was to increase the effectiveness of pharmacological correction of ulcerative colitis with dalargin.

Materials and Methods: Ulcerative colitis was simulated by replacing drinking water with a 5% solution of dextran sodium sulfate for 5 days. The mice were killed on the 5th, 7th and 28th days; the colon was removed. Dalargin was dissolved in a 0.9% sodium chloride solution, injected subcutaneously daily at a dose of 100 μg/kg once a day for 7 days. Sulfasalazine was used as a reference drug.

Results and Discussion: The dalargin administration decreased disease activity index, pathological colonic shortening, prevalence of ulcers and infiltrates in colon, increased goblet cell number, acid and neutral mucins concentrations in mice with ulcerative colitis. The mechanisms of pharmacological dalargin effect include: drop in proinflammatory interleukins (IL-1β, IL-6, IL-17) and matrix metalloproteinases concentration, TGF-β, arising in anti-inflammatory interleukins, epidermal growth factor, inhibitor of matrix metalloproteinases-2 content. Dalargin corrected phagocytes activity and leukocyte indices. Dalargin action in ulcerative colitis is higher than that of sulfasalazine.

Conclusion: Pharmacological dalargin effect on ulcerative colitis development was explained by its effect on opioid μ-receptors on macrophages, neutrophils, and lymphocytes of the colonic wall. The therapeutic action involves antioxidant effect and endothelial dysfunction correction.

Graphical abstract



Keywords

dalargin, goblet cells, interleukins, matrix metalloproteinases, mucins, ulcerative colitis

Introduction

Ulcerative colitis (UC) is a chronic disease of the colon, characterized by immune inflammation of its mucous membrane (Shelygin et al. 2023). Damage to the colon in UC is diffuse, including the obligatory involvement of the rectum, usually limited to the mucous membrane, but inflammation spreads to the deep layers of the colonic wall in acute severe UC (Shelygin et al. 2023). UC is a widespread chronic disease of the colon with frequent relapses, affecting mainly people of active age and significantly reducing the life quality of patients. According to epidemiological studies in the Russian Federation, the prevalence of UC is 20-23 per 100,000 of population with a tendency to increase the frequency of severe complications of the disease (Belousova et al. 2018). The UC incidence is the same in men and women in Russia and some other countries (Belousova et al. 2018; Belousova et al. 2023). UC most commonly afflicts adults aged 20-30, although in some countries, a second peak of incidence has been noted in patients aged 60-70 (Belousova et al. 2018; Belousova et al. 2023; Shelygin et al. 2023). According to the National Register, the average age of onset of the disease in Russia is 36.1 years, and no significant changes in this indicator have been noted over the past seven years (Belousova et al. 2018). Inflammatory bowel diseases (UC and Crohn's disease) are more common in the urban population of industrialized countries (Belousova et al. 2023). The reasons for this fact are related to the nutritional habits of the population of these countries, as well as a high level of diagnosis of colonic diseases. UC is one of the most severe diseases of the gastrointestinal tract, as it affects mainly young people and is accompanied by frequent relapses and the development of severe complications requiring surgical treatment (Belousova et al. 2023).

The UC etiology and pathogenesis remain insufficiently studied, despite the high social UC significance and a large number of investigations focused on this disease (Akinshina et al. 2019; Belousova et al. 2023; Le Berre et al. 2023). It has been established that the UC development is associated with the complex effect of genetic and environmental factors, disorders of innate and acquired immunity, changes in the composition of the colonic microflora (Luo et al. 2019; Shelygin et al. 2023).

Experimental studies have proven that the leading mechanism for the UC development is disturbance in the barrier function of the colon, leading to the penetration of pathogenic microflora and the development of chronic inflammation in the colon (Akinshina et al. 2019; Le Berre et al. 2023). Although therapeutic options for UC are expanding, 10-20% of patients still require proctocolectomy due to a drug-resistant disease. Precise and personalized therapy may be the key to overcoming this therapeutic ceiling (Polikarpova et al. 2022). The effectiveness of the existing methods of treatment remains low, despite a significant number of studies to study various UC aspects (Le Berre et al. 2023; Shelygin et al. 2023). Considering the above, the search for new highly effective methods for UC treating is an urgent task aimed at solving an important medical and social problem.

Dalargin is an analogue of leu-enkephalin, proposed as a pharmacological drug for the gastric and duodenal ulcers treatment (Bulgakov 2018). It was previously shown that dalargin normalizes microcirculation and lymph dynamics in the inflammation zone, activates macrophages, increases the number of contacts between macrophages and fibroblasts, and enhances the synthesis of collagen and DNA in the epithelium (Bulgakov 2018). Dalargin high medicinal efficacy in various forms of pathology and its ability to stimulate tissue regeneration have been established (Zabrodin 2016). Dalargin has a number of pharmacological effects, including antioxidant, membrane-stabilizing and immunomodulatory effects (Dontsov 2015; Lishmanov et al. 2012; Bulgakov 2018; Platonova et al. 2018).

The aim of the study: to increase the effectiveness of pharmacological correction of ulcerative colitis by using dalargin.

Materials and Methods

Drugs

The pharmacological effects of dalargin (Scientific and Production Association "Microgen", Russian Federation) in experimental UC in mice were investigated. The lyophilized powder of the drug was dissolved in 1 mL of physiological solution according to the instructions. Dalargin was administered to mice with UC subcutaneously at a dose of 100 mcg per 1 kg of body weight in a volume of 0.1 mL for 7 days, starting from the day of UC simulation. Sulfasalazine (KRKA, Slovenia) was used as a reference drug at a dose of 200 mg per 1 kg of body weight intragastrically in the form of a suspension in physiological solution in a volume of 0.3 mL for 7 days (Motov et al. 2021). Sulfasalazine is widely administered in clinical practice and is used as a reference drug in experimental studies of UC. Mice in the control group were injected with saline: one subgroup – subcutaneously in a volume of 0.1 mL, and the other subgroup –intragastrically in a volume of 0.3 mL, for 7 days. Dextran sodium sulfate (DSS) (Mr=40000) was purchased from PanReac-AppliChem and neoFroxx GmbH (Germany).

Animals

208 male Balb/C mice weighing 20-23 g were purchased from Stolbovaya branch of the Federal State Budgetary Institution of Science "Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency". The animals were quarantined in the vivarium of Kursk State Medical University for at least 10 days. Mice without external signs of the disease were used in the experiments, which were performed from 9.00 to 13.00 each day. All mice were housed 7/cage and were fed standard laboratory chow in an animal room with 12 h dark/light cycles at a constant temperature of 20±5°C. Mice were individually marked with small incisions on the ears. All animal experiments were conducted in the Laboratory of Preclinical Trials of Drugs of the Research Institute of Experimental Medicine of Kursk State Medical University under guidelines of humane treatment of laboratory animals (Lipatov et al. 2019a; Lipatov et al. 2019b), the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 and the Rules of Good Laboratory Practice in the Russian Federation (order of the Ministry of Health of the Russian Federation No. 199n dated April 1, 2016). The experiments were approved by the Regional Ethics Committee (REC) (Minutes No. 1 of March 4 2023).

Experimental design

The investigation was carried out on 208 male Balb/C mice, 12 of which were intact. UC was simulated in 196 remaining mice. All UC animals were randomly divided into 4 experimental groups: 1) control No. 1 (UC+saline subcutaneously, n=48); 2) control No. 2 (UC+saline intragastrically, n=48); 3) experimental No. 1 (UC+dalargin solution, n=48); 3) experimental No. 2 (UC+sulfasalazine, n=48). Eight mice from each group were killed by cervical dislocation under the chloral hydrate anesthesia (Macklin, China) on the 5th, 7th, and 28th days. It was established there were neither clinical nor morphological differences between Balb/C mice with experimental UC treated with saline in the indicated ways at all stages of the experiment. The control group included 12 mice which were treated with saline administered subcutaneously and 9 mice with saline administered intragastrically, and therefore 4 mice treated with saline subcutaneously and 3 mice treated with saline intragastrically were killed on each day in the experiments to measure IL-1β, IL-4, IL-6, IL-10, IL-17, MMP-2, MMP-9, TIMP-2, TGF-β, EGF considering that dalargin and sulfasalazine were administered to animals in different ways. Saline was administered once a day for 7 days from the beginning of UC simulation in a volume of 0.1 mL subcutaneously or 0.3 mL intragastrically.

Research Methods

UC simulation in Balb/C laboratory mice was completed using the traditional method – replacing drinking water with a 5% solution of dextran sodium sulfate (DSS) (Mr=40000; PanReac-AppliChem, Germany and neoFroxx GmbH, Germany) in boiled water for 5 days (Khomyakova et al. 2013). It was previously shown that a 5% DSS solution causes the development of experimental colitis in Balb/C mice. Animals are killed by cervical dislocation under the chloral hydrate anesthesia (Macklin, China) on the 5th, 7th (acute colitis), and 28th (chronic colitis) days. In mice with DSS-induced colitis, the disease activity index (DAI) was calculated using 3 parameters: weight loss, stool consistency, and rectal bleeding. The colon was removed and its length in cm was measured on the 5th, 7th, and 28th days.

The distal colon was isolated and placed in a buffered neutral 10% formaldehyde solution for 1-2 weeks. Sections of the colonic wall with a thickness of 5-6 microns were made, which were stained with

hematoxylin-eosin (BioVitrum, Russia), alcian blue pH = 1.0 according to Mowry to identify highly sulfated acidic (HSA) mucins and Schiff's reagent to determine neutral mucins (BioVitrum, Russia). Histological and morphometric analyses of the tissue structure were carried out.

Light microscopy was performed on a Nicon Eclipse Ni microscope using NIS Elements AR software. The intensity of the ulcerative process was assessed on longitudinal sections stained with hematoxylin and eosin. The prevalence of colon mucosal ulcers and inflammatory infiltrates in the lamina propria was measured as a function of the length of the muscularis lamina. The number of goblet cells (GC) was assessed from photographs of alcian blue-stained sections per crypt. The content of HSA and neutral mucins was assessed by the intensity of GC staining with alcian blue and Schiff's reagent after treatment with periodic acid, respectively. Color intensity was calculated as the average decimal logarithm of the ratio of the background brightness to the one of the object point in the photograph.

The medial colonic section was isolated and opened with a longitudinal incision along the edge of the mesentery attachment, washed with phosphate-buffered saline (pH = 7.4, 0.01 M) and tissue (70 mg) was homogenized in a Potter-Elweheim homogenizer for 10 minutes. The content of interleukin (IL)-1β, IL-4, IL-6, IL-10, IL-17, matrix metalloproteinase (MMP)-2, MMP-9, tissue inhibitor of matrix metalloproteinases (TIMP)-2, transforming growth factor (TGF)-β, epithelial growth factor (EGF) was determined in the homogenate of the medial colon by enzyme-linked immunosorbent assay (ELISA) using standard kits from Cloud-Clone Corp. (China) on an automatic enzyme immunoassay analyzer "Lazurit" (Dynex Technologies, USA) according to the attached instructions. Blood was collected from the animals, and smears were prepared by applying a drop of blood to a glass slide. After fixation, the smears were stained according to Romanovsky-Giemsa. The relative number of leukocytes types (leukocyte formula) was calculated. The following hematological indices were calculated: 1) Krebs index (ratio of neutrophils to lymphocytes); 2) Bredeck coefficient (ratio of lymphocytes to band neutrophils); 3) neutrophil-monocyte ratio index (NMRI); 4) lymphocyte-monocyte ratio index (LMRI); 5) lymphocyte-to-eosinophil ratio index (LERI); 6) leukocyte index (LI) – the ratio of lymphocytes to neutrophils (Supilnikov and Shabalin 2018).

The phagocytic activity of peripheral blood neutrophils was determined by the traditional method (Kutepov et al. 2019). The phagocytic index (PI) was determined – the number of actively phagocytic neutrophils per 100 cells; the phagocytic number (PN) is the average number of microbial bodies captured by one phagocytic neutrophil, and the opsonophagocytic index (OPI) is the average number of absorbed microbial bodies per 100 neutrophils.

Statistical data processing

Statistical processing of the obtained results included: 1) determination of normality of distribution using the Shapiro-Wilk test; 2) assessment of homogeneity of variances using Levene's test; 3) testing of statistical hypotheses using the nonparametric Mann-Whitney U test. The material is presented as median (Me), lower (Ql) and upper (Q3) quartiles. During statistical analysis, the null hypothesis was rejected at $p \leq 0.05$. Factor analysis by the method of principal components was used to determine statistical correlations. The indicators were considered to be correlated if the factor loading was more than 0.7. Statistical processing was carried out using Statistica 10 and Statistica 13 software (USA).

Results and Discussion

The experimental UC development in Balb/C mice was accompanied by a decrease in weight on the 5th day by 8.7% in both control groups compared to the beginning of the experiment, stool disturbances (diarrhea – 50%, loose stool – 50%), and the appearance of blood in feces. The motor activity decrease, periodic occurrence of a painful posture (arched back), and coat changes (disheveled and dirty) were noted in the animals behavior investigation. It was found that the colonic length decreased by 31.0-32.1% compared to intact mice (p=0.0009). Weight loss in animals of control groups with UC increased and amounted to 13.0% on the 7th day. The diarrhea frequency, on the contrary, decreased. UC mice continued to have low motor activity, periodic painful posture, and coat abnormalities. The colonic length decreased slightly – by 33.2-34.7%, compared with the intact group (p=0.0009).

The administration of dalargin solution in UC mice led to a significant decrease in the severity of rectal bleeding in points by 50.0% (p=0.0406) compared to control group No. 1 on the 5th of the experiment. The colonic length is greater by 31.2% than that of control mice (p=0.0009) (Table 1). Other indices, including DAI, did not change. DAI decreased by 28.6% (p=0.0463) and the colonic length increased by 23.5% (p=0.0009) on the 7th day of UC simulation in mice treated with dalargin solution.

The administration of reference drug sulfasalazine in UC mice resulted in a significant increase in the colonic length by 11.1% (p=0.0014) compared to control group No. 2 on the 5th day of the experiment. The studied indices changed significantly: weight loss was less by 30.0% (p=0.0009), DAI was less by 20.0% (p=0.0406), and the colonic length increased by 9.1% (p=0.0074) on the 7th day.

Weight loss and loose stools were observed in 2-3 animals out of 8 in all groups; there were no significant differences in the colonic length on the 28th day, with the chronic UC development. The colonic length in animals treated with dalargin was longer by 16.2% (p=0.0009) and by 15.9% (p=0.0019) than in mice that were administered with sulfasalazine on the 5th and 7th days, respectively.

Simulation of DSS-induced UC in Balb/C mice led to the formation of ulcers of the colon mucosa and infiltrates in the lamina propria of the mucosa (LPM) and submucosal layer. The median prevalence of mucosal ulcers on the 5th day of the experiment was 11.4-11.7% in control groups No. 1 and No. 2. The rate increased significantly by 24.3% and 24.2% on the 7th day, respectively. The presence of ulcers was noted in all animals, but the median prevalence had very low values of 1.1% and 0.9%, respectively in chronic UC mice. Similar dynamics were established in analyzing the infiltrates prevalence in the LPM. The indices in the two control groups were 26.9% and 25.8%, respectively on the 5th day. The infiltrates prevalence of LPM peaked on the 7th day: 52.9% and 54.6%, respectively. The prevalence rate was 24.6% and 26.4%, respectively in chronic UC mice.

Table 1. Effect of dalargin and sulfasalazine on disease activity index, colon length, prevalence of ulcers and infiltrates in mice with ulcerative colitis, Me [Q1; Q3]

№	Experimental group	Days	Disease activity index, points	Colon length, cm	Ulcer prevalence, %	Infiltrates prevalence, %
1	Intact			13.7 [13,5; 14,1]		
2	Control group No. 1 (experimental UC + saline subcutaneously)	5	6.1 [4.0; 8.0]	9.3 [8.6; 10.0]^{x} p=0.0009	11.7 [10.4; 12.6]	26.9 [22.8; 30.1]
		7	7.0 [5.0; 8.0]	9.2 [8.3; 9.4]^{x} p=0.0009	24.3 [19.8; 26.3]	52.9 [44.6; 61.6]
		28	-	13.2 [12.9; 13.6]	1.1 [0.7; 1.4]	24.6 [21.0; 27.3]
3	Control group No. 2 (experimental UC + saline in the stomach)	5	6.0 [4.5; 8.0]	9.5 [9.0; 9.8]^{x} p=0.0009	11.4 [10.3; 12.0]	25.8 [22.0; 29.0]
		7	6.0 [5.0; 7.0]	9.0 [8.8; 9.3]^{x} p=0.0009	24.2 [21.2; 27.7]	54.6 [49.1; 60.6]
		28	-	13.4 [13.0; 14.0]^{x}	0.9 [0.6; 1.4]	26.4 [22.4; 29.0]
4	Experimental UC + dalargin at the dose 100 mcg/kg subcutaneously	5	4.0 [2.5; 6.0]	12.2 [11.9; 12.7]*^{1} *p=0.0009 ^{1}p=0.0009	6.4 [4.0;9.2]* p=0.0039	20.0 [17.1; 22.9]*^{1} *p=0.0136
		7	5.0 [3.0; 5.5]* p=0.0463	11.3[11.0; 12.1]*^{1} *p=0.0009 ^{1}p=0.0019	9.8 [8.5; 10.9]*^{1} *p=0.0009 ^{1}p=0.0009	29.5 [25.8; 33.5]* p=0.0009
		28	-	13.6 [13.2; 14.2]	0.3 [0.0; 0.7]* p=0.0009	13.1 [10.0; 16.2]*^{1} *p=0.0009 ^{1}p=0.0239
5	Experimental UC + sulfasalazine at the dose 200 mg/kg in the stomach	5	5.0 [4.0; 7.5]	10.5 [10.2; 11.1]* p=0.0014	6.1 [4.3; 7.9]* p=0.0009	25.4 [22.7; 28.1]
		7	5.0 [4.0; 5.0]* p=0.0406	9.8 [9.4; 10.3]* p=0.0074	11.8 [10.8; 12.7]* p=0.0009	31.3 [27.1; 36.9]* p=0.0014
		28	-	13.1[12.6; 13.6]	0.4 [0.0; 0.9]	19.1 [17.6; 20.7]* p=0.0054

***Note*:** p<0.05 compared to the indices: ^{x} – intact group; * – control group; ^{1} – animal treated with sulfasalazine.

Dalargin administration had a therapeutic effect on the UC development. A decrease in the mucosal ulcers and LPM infiltrates prevalence was shown by 45.3% (p=0.0039) and 26.2% (p=0.0136), respectively on the 5th day of the experiment. The studied indices decreased by 2.5 times and by 44.2% (p=0.0009) on the 7th day, respectively. Only three out of eight mice with chronic UC (the 28th day) were found to have mucosal ulcers, with the median lower by 4.0 times than in the control group (p=0.0009). The infiltrates prevalence in LPM decreased by 46.7% (p=0.0009).

Reference drug sulfasalazine also had a therapeutic effect on the UC course. The ulcers prevalence decreased by 46.5% (p=0.0009) on the 5th day only. The ulcers prevalence was lower by 2.1 times (p=0.0009), and the LPM infiltrates prevalence was 43.0% lower than in the control group (p=0.0014) on the 7th day. Only 3 of the 8 mice with chronic UC that received sulfasalazine had mucosal ulcers, but the median did not differ significantly from that in the control group. The infiltrates prevalence was 27.7% lower than in the control group (p=0.0054). The ulcers prevalence on the 7th day and the infiltrates prevalence on the 5th and the 28th days in the UC+dalargin group were lower than in the UC+sulfasalazine group (p=0.0054-0.0239).

UC simulation in mice was accompanied by a decrease in the number of goblet cells (GC) by 32.7-33.2% on the 5th day, by 47.1-47.6% on the 7th day, by 15.4-15.9% on the 28th day of the experiment (p=0.0024) in both control groups (Table 2). First of all, a decrease in the number of GC at the bottom of crypts was observed. The GC amount is higher in mice with chronic UC than in animals with an acute process (5-7 days) by 25.7-61.5% (p=0.0009). There was a decrease in the number of HSA (by 3.43-3.75 times) and neutral (by 36.3%) mucins in the colon mucosa on the 5th day, as well as by 3.64-3.87 times and by 38.2- 39.3% on the 7th day of the experiment, respectively (p=0.0024). The mucins amount was higher in animals with chronic UC than in mice with an acute process, but at the same time lower when compared to the intact group (highly sulfated acid (HSA) – by 42.5-43.3%, and neutral – by 27.5-29.4 % (p=0.0024)).

Dalargin administration caused an increase in the GC number in UC mice on the 5th-7th days of the experiment compared to control animals, but not on the 28th day. The increase in this index was by 3.6% on the 5th day, and on the 7th day – by 19.3% in dalargin administration. Dalargin led to an increase in the amount of both types of mucins in mice with UC compared to control group No. 1. Thus, the increase in the number of HSA mucins was by 50.0% (p=0.0239) on the 5th day, by 54.8% (p=0.0136) – on the 7th day of the experiment, and the neutral mucins increase was by 6.2% (p=0.0136) on the 5th day and by 7.9% (p=0.0009) on the 7th day in dalargin administration.

Table 2. Effect of dalargin and sulfasalazine on the number of goblet cells in crypts, the content of highly sulfated acid and neutral mucins in the colon of mice with experimental ulcerative colitis, Me [Q1; Q3]

№	Experimental group	Experiment duration, days	Goblet cells, n	lg_{10} content of neutral mucins	lg_{10} content of highly sulfated acid mucins
1	Intact animals		20.8 [20.7; 20.9]	1.0 [1.0; 1.1]	1.2 [1.1; 1.4]
2	Control group No. 1 (experimental UC + saline subcutaneously)	5	14.0 [13.9; 14.1]^{x} p=0.0024	0.7 [0.7; 0.7]^{x} p=0.0024	0.3 [0.3; 0.4]^{x} p=0.0024
		7	10.9 [10.7; 11.0]^{x} p=0.0024	0.6 [0.6; 0.6]^{x} p=0.0024	0.3 [0.3; 0.4]^{x} p=0.0024
		28	17.6 [17.3;17.9]^{x} p=0.0024	0.7 [0.7; 0.7]^{x} p=0.0024	0.7 [0.6; 0.8]^{x} p=0.0024
3	Control group No. 2 (experimental UC + saline in the stomach)	5	13.9[13.7; 14.3]^{x} p=0.0024	0.7 [0.6; 0.7]^{x} p=0.0024	0.4 [0.3; 0.4] ^{x} p=0.0024
		7	11.0 [10.8; 11.2]^{x} p=0.0024	0.6 [0.6; 0.6]^{x} p=0.0024	0.3 [0.3; 0.4]^{x} p=0.0024
		28	17.5 [17.4; 17.7]^{x} p=0.0024	0.7 [0.7; 0.8]^{x} p=0.0024	0.7 [0.5; 0.8]^{x} p=0.0024
4	Experimental UC + dalargin at the dose 100 mcg/kg subcutaneously	5	14.5 [14.3; 14.6]* p=0.0011	0.7 [0.7; 0.7]* p=0.0136	0.5 [0.4; 0.6]* p=0.0239
		7	13.0 [12.9; 13.2]*^{1} *p=0.0009; ^{1}p=0.0009	0.7 [0.7; 0.7]*^{1} *p=0.0019; ^{1}p=0.0019	0.5 [0.4; 0.6]* p=0.0136
		28	18.0 [17.7; 18.6] p=0.0587	0.8 [0.8; 0.8]^{1} *p=0.0520; ^{1}p=0.0023	0.8 [0.7; 0.8] p=0.3184;
5	Experimental UC + sulfasalazine at the dose 200 mg/kg in the stomach	5	14.3 [14.1; 14.5]* p=0.0011	0.7 [0.6; 0.7]* p=0.0101;	0.5 [0.4;0.6] p=0.0831
		7	12.1 [11.9; 12.3]* p=0.0009	0.6 [0.6; 0.6] p=0.4623	0.5 [0.4;0.6]* p=0.0209
		28	18.0 [17.7; 18.1] p=0.0587	0.8 [0.7; 0.8]* p=0.0136	0.7 [0.6; 0.8] p=0.8337

Note: p<0.05 compared to the indices: ^{x} – intact group; * – control group; ^{1} – animal treated with sulfasalazine.

Sulfasalazine administration to UC mice increased the amount of GC by 2.9% (p=0.0011) compared to control group No. 2 on the 5th day of the experiment. The increase was by 10.0% (p=0.0009) on the 7th day. The increase in the neutral mucins content in the UC+sulfasalazine group was by 3.1% on the 5th and 28th days.

There was an increase in the GC amount by 7.4% (p=0.0009), in the content of neutral mucins in mice of the UC+dalargin group – by 7.9% (p=0.0019) on the 7th day, and by 8.3% (p=0.0023) on the 28th day compared to the UC+sulfasalazine group. There was an increase in the pro-inflammatory IL content in the colonic wall in mice with acute experimental UC (Table 3). The increase in the IL-1β and IL-6 concentrations was maximum on the 5th day of UC development (by 3.94 and 6.75 times, respectively, p=0.0107). The concentration of IL-17 reached its maximum value on the 7th day of UC development and was higher by 6.21 times compared to the intact group (p=0.0107). Changes in the concentration of anti-inflammatory cytokines IL-4 and IL-10 were multidirectional. The IL-4 content decreased on the 5th and 7th days of the experiment by 46.5% and by 2.35 times, respectively (p=0.0107); however, the IL-10 concentration increased by 4.15 and 5.75 times, respectively (p=0.0107). The IL-6 concentration was only significantly higher by 2.5 times in chronic UC than in intact mice (p=0.0107).

Dalargin administration had an immunomodulatory effect on the pro- and anti-inflammatory cytokines content in the colonic wall. A decrease in the pro-inflammatory cytokines concentration was found on the 5th day: IL-1β – by 35.8% (p=0.0022), IL-6 – by 55.6% (p=0.0060), and IL-17 – by 42.1% (p=0.0022). The IL-1β content decreased by 41.2% (p=0.0022) and IL-17 – by 48.8% compared to the control group (p=0.0022) on the 7th day. . Dalargin administration caused an increase in the IL-4 and IL-10 content on the 5th and 7th days of the experiment in the colonic wall. Thus, the IL-4 concentration was higher by 29.1% (p=0.0040), and IL-10 concentration was higher by 2.19 times (p=0.0022) on the 5th day compared to the control group. The IL-4 content was higher by 50.0% (p=0.0022), and IL-10 concentration was higher by 59.6% (p=0.0022) in animals treated with dalargin after 7 days. Dalargin administration affected the concentrations of IL-17 and IL-10 only.

UC simulation in Balb/C mice was accompanied with an increase in the MMP-2, MMP-9 and TIMP-2 content in the homogenate of the medial colon on the 5th day of the experiment by 16.1, 21.8 and 20.7 times, respectively (p=0.0107) compared to intact animals (Table 4). There was a decrease in the MMP-2 and TIMP-2 concentrations by 37.9% and 20.0%, respectively on the 7th day, compared to the previous observation period. On the contrary, MMP-9 content increased by 11.5%. The MMP-2, MMP-9, TIMP-2 concentrations decreased significantly in chronic colitis.

Dalargin administration in UC mice caused a significant decrease in the MMP-2, MMP-9 and TIMP-2 content on the 5th and 7th days of the experiment: the MMP-2 concentration decreased by 6.3 and 3.9 times, MMP-9 – by 4.1 and 5.6 times, and TIMP-2 – by 46.7% and 35.4%, respectively, on the 5th and 7th days after the starting of UC simulation compared to the control group (p=0.0022). There were no significant differences in the MMP-2 and MMP-9 concentrations on the 28th day. The TIMP-2 content was less by 3.7 times. Sulfasalazine administration in mice with UC decreased the MMP-2 and TIMP-2 contents in the homogenate of the medial colon by 3.1 times (p=0.0022) and by 28.3% (p=0.0022) on the 5th day, respectively, compared to animals of the control group. No changes in the MMP-9 concentration were detected during this period.

Table 3. Effect of dalargin and sulfasalazine on the pro- and anti-inflammatory interleukins content in the colon of mice with experimental ulcerative colitis, Me [Q1; Q3]

Groups	Days	IL-1β content (pg/mg tissue protein)	IL-6 content (pg/mg tissue protein)	IL-17 content (pg/mg tissue protein)	IL-4 content (pg/mg tissue protein)	IL-10 content (pg/mg tissue protein)
Intact		34.0 [31.6; 56.0]	1.2 [0.9; 1.4]	3.9 [1.2; 7.8]	56.5 [54.0; 60.0]	3.9 [1.2; 7.8]
Control group (experimental UC + saline)	5	134.0 [120.0; 142.0] [x] p=0.0107	8.1 [5.8; 8.3][x] p=0.0107	22.8 [20.1; 24.2][x] p=0.0107	30.2 [28.0; 32.0][x] p=0.0107	16.1 [14.4; 18.9][x] p=0.0107
	7	114.0 [106.0; 119.0] [x] p=0.0107	3.6 [3.4; 4.1] [x] p=0.0107	24.2 [23.2; 28.6][x] p=0,0107	24.0 [21.0; 28.0] [x] p=0.0107	22,3 [18,4; 24,3][x] p=0.0107
	28	61.4 [58.1; 79.0] p=0.1082	3.0 [2.8; 3.6] [x] p=0.0107	9.9 [6.1; 11.3] p=0.1082	64.0 [40.0; 86.0] p=0.7768	4.2 [2.0; 12.2] p=0.3951
Experimental UC + dalargin at a dose of 100 mcg/kg subcutaneously	5	86.0 [76.0; 86.3]* p=0.0022	3.6 [3.4; 5.7]* p=0.0060	13.2 [12.2; 14.4]*[1] *p=0.0022, [1]p=0.0253	39.0 [34.0; 46.0]* p=0.0040	35.2 [31.8; 36.4]*[1] *p=0.0022, [1]p=0.0215
	7	67.0 [48.0; 69.0]* p=0.0022	3.3 [3.2; 3.9] p=0.3711	12.4 [11.8; 14.3]* p=0,0022	36.0 [32.0; 44.0]* p=0.0022	36.0 [28.0; 51.1]* p=0.0022
	28	58.0 [41.0; 67.0] .p=0.1599	2.4 [2.2; 2.9] p=0.1417	5.4 [2.1; 7.1]* p=0.0409	55.1 [50.0; 56.0] p=0.7015	28.5 [3.9; 32.5]* p=0.0409
Experimental UC + sulfasalazine at a dose of 200 mg/kg in the stomach	5	95.0 [82.0; 95.3]* p=0.0049	4.6 [4.0; 6.4]* p=0.0106	15.2 [14.1; 18.1]* p=0.0022	34.0 [32.0; 38.0] p=0.0639	24.6 [24.1; 26.8]* p=0.0106
	7	71.6 [69.0; 78.2]* p=0.0073	3.6 [3.6; 3.9] p=0.7983	14.1 [12.9; 18.9]* p=0.0022	32.0 [30.0; 38.0]* p=0.0049	29.3 [26.9; 30.4]* p=0.0049
	28	51.1 [36.0; 64.0]* p=0.0409	2.3 [2.0; 2.4]* p=0,0088	7.4 [5.3; 8.4] p=0,1102	52.0 [45.0; 56.8] p=1.00	15.2 [3.9; 18.2] p=0.2502

Notes: p<0.05 compared to the indices: [x] – intact group; * – control group; [1] – animal treated with sulfasalazine.

Table 4. Effect of dalargin and sulfasalazine on the matrix metalloproteinases-2, -9 and tissue inhibitor of metalloproteinases-2 contents in the colon of mice with experimental ulcerative colitis, Me [Q1; Q3]

Groups	Days	Matrix metalloproteinase – 2 (ng/mg tissue protein)	Matrix metalloproteinase – 9 (ng/mg tissue protein)	Tissue inhibitor of matrix metalloproteinase – 2 (ng/mg tissue protein)
Intact		1.8 [1.2; 2.4]	0.6 [0.3; 0.7]	0.3 [0.3; 0.6]
Control group (experimental UC + saline)	5	29.0 [25.4; 31.0][x] p=0.0107	13.1 [12.6; 14.0][x] p=0.0107	6.0 [5.4; 6.8][x] p=0.0107
	7	18.0 [9.9; 22.0][x] p=0.0107	14.6 [14.4; 15.2][x] p=0.0107	4.8 [3.9; 6.4][x] p=0.0107
	28	1.3 [1.3; 1.6] p=0.6366	0.8 [0.7; 0.9] p=0.1859	2.3 [1.6; 2.8][x] p=0.0107
Experimental UC + dalargin at a dose of 100 mcg/kg subcutaneously	5	4.6 [4.4; 5.1]*[1] *[1]p=0.0022	3.2 [2.3; 4.6]*[1] *[1]p=0.0022	3.2 [3.0; 3.7]*[1] *[1]p=0.0022
	7	4.6 [4.2; 5.1]*[1] *[1]p=0.0022	2.6 [2.4; 2.9]*[1] *[1]p=0.0022	3.1 [2.8; 3.6]* p=0.0022
	28	1.6 [0.9; 21] p=0,8480	0.8 [0.6; 0.9] p=0.3067	0.6 [0.5; 0.7]* p=0.0033
Experimental UC + sulfasalazine at a dose of 200 mg/kg in the stomach	5	9.4 [8.4; 9.5]* p=0.0022	12.3 [11.0; 13.2] p=0.3067	4.3 [3.8; 4.6]* p=0.0022
	7	8.4 [7.6; 9.3]* p=0.0049	4.9 [4.3; 5.0]* p=0.0022	2.8 [2.8; 3.3]* p=0.0022
	28	1.3 [0.8; 1.8] p=1.00	0.9 [0.5; 1.1] p=0.4433	1.4 [0.9; 1.8]* p=0.0409

Note: p<0.05 compared to the indices: [x] – intact group; * – control group; [1] – animal treated with sulfasalazine.

The MMP-2 content was less 2.1 times (p=0.0049), MMP-9 concentration – by 3.0 times (p=0.0022), TIMP-2 content – by 41.7% (p=0.0022) than in the control group on the 7th day of the experiment. The TIMP-2 concentration was less by 39.1% (p=0.0409) than in mice of the control group in chronic UC. The dalargin effects were found to be significantly higher compared to those of sulfasalazine: on the 5th and 7th days, the MMP-2 content was less by 2.0 times and by 45.2% in mice treated with dalargin compared to UC+sulfasalazine group (p=0.0022). The MMP-9 content in mice treated with dalargin was significantly less than in the UC+sulfasalazine group: on the 5th day – by 3.8 times (p=0.0022), and on the 7th day – by 46.9 % (p=0.0033). Lower TIMP-2 concentrations in mice treated with dalargin were observed only on the 5th day of the experiment – by 25.6% (p=0.0022). Mice with chronic UC treated with dalargin showed a 2.2-fold decrease in TIMP-2 content on the 28th day (p=0.0181).

UC simulation in male Balb/C mice was accompanied with an increase in the TGF-β content in the homogenate of the medial colon on the 5th and 7th days of the experiment (acute UC) – by 4.3 and 3.9 times, respectively (p=0.0107) compared to the intact group (Table 5). The EGF content on the 7th and 28th days of the experiment increased by 29.6% and 3.4 times, respectively, compared to intact mice (p=0.0107). Dalargin administration caused a decrease in TGF-β levels on the 5th and 7th days of the experiment compared to those in the control group by 49.4% and 45.8% (p=0.0022), respectively. The concentration of TGF-β was higher in chronic UC than in the control group – by 40.4% (p=0.0212). An increase in EGF concentration in mice with UC treated with dalargin was also found throughout the experiment: on the 5th day – by 91.7% (p=0.0022), on the 7th day – by 2.4 times (p=0.0022), and on the 28th day – by 2.9 times (p=0.0022). A TGF-β levels decrease was shown on the 5th and 7th days after the start of UC simulation by 36.4% and 48.5%, respectively (p=0.0022) in the group UC+sulfasalazine, and EGF content on the 7th day – only by 40.2 % (p=0.0033). Comparing the effects of dalargin and sulfasalazine on the TGF-β and EGF contents, it was found the EGF concentration only was significantly higher in animals treated with dalargin throughout the experiment.

An increase in the phagocytic activity of peripheral blood neutrophils was found on the 5th and 7th days of the experiment in Balb/C mice with experimental UC: an increase in PI was by 29.7-52.2% (p=0.0019), PN – by 65.5% (p=0.0009), and OPI – by 2.2-2.3 (p=0.0009) times in animals of control groups compared to intact ones. Mice with acute colitis showed an increase in PI by 57.1% (p=0.0009), PN – by 69.0-75.9% (p=0.0009), and OPI – by 2.7- 2.8 (p=0.0009) times after 7 days of the experiment. No significant differences were found on the 28th day in animals with chronic UC.

Dalargin administration in mice with experimental UC had a corrective effect on the phagocytic activity of neutrophils, which was manifested by a decrease in PI by 32.4%, in PN – by 16.7%, and in OPI – by 39.8% on the 5th day and by 22.4%, 17.6%, 36.6%, respectively, on the 7th day (p=0.0009-0.0063). Dalargin did not have a statistically significant effect on the phagocytic activity of neutrophils on the 28th day. Sulfasalazine administration caused a decrease in PI by 14.6% (p=0.0046), OPI – by 28.4% (p=0.0046), but had no affect on PN (p>0.05) on the 5th day of observation. A decrease in all 3 studied parameters was found in UC mice treated with sulfasalazine: in PI – by 16.7% (p=0.0016), in PN – by 10.2% (p=0.0028), and in OPI – by 25.3% (p=0.0009) on the 7th day of the experiment. There were no changes in the studied parameters on the 28th day in mice of UC + sulfasalazine group (p>0.05). No statistically significant differences were found when comparing the effects of dalargin and sulfasalazine on PI, PN and OPI (p>0.05).

Table 5. Effect of dalargin and sulfasalazine on the concentrations of transforming growth factor-β and epidermal growth factor in the homogenate of the medial colon, Me [Q1; Q3]

Indicators Group	Duration of experiment, days	Content of TGF-β in colon homogenate (pg/mg tissue protein)	Content of EGF in colon homogenate (pg/mg tissue protein)
Intact		373.0 [203.0; 520.0]	24.0 [22.4; 27.5]
Control group (UC+physiological solution)	5	1620.0 [1550.0; 2510.0]^{x} p=0.0107	24.0 [24.0; 27.0] p=0.0890
	7	1440.0 [1410.0; 1500.0]^{x} p=0.0107	31.1 [25.0; 38.0]^{x} p=0.0107
	28	520.0 [430.0; 580.0] p=0.2193	82.0 [71.0; 91.0]^{x} p=0.0107
Group ulcerative colitis+dalargin at a dose of 100 mcg/kg	5	820.0 [801.0; 950.0]* p=0.0022	46.0 [38.8; 51.0]*^{1} *p=0,0022, ^{1}p=0.0049
	7	780.0 [722.0; 800.0]* p=0.0022	74.0 [73.0; 76.7]*^{1} *^{1}p=0.0022
	28	730,0 [540,0; 760,0]* p=0,0212 p=0,0845	241.0 [126.0; 284.0]*^{1} *^{1}p=0.0022
Group ulcerative colitis+sulfasalazine at a dose of 200 mg/kg	5	1030.0 [841.0; 1190.0]* p=0.0022	23.5 [7.0; 26.0] p=0.3067
	7	742.0 [730.0; 788.0] p=0.0022	43.6 [39.9; 49.6] p=0.0033*
	28	574.0 [490.0; 720.0] p=0,6093	73.1 [59.9; 78.7] p=0.3067

***Notes*:** p<0.05 compared to the indices: ^{x} – intact group; * – control group; ^{1} – animal treated with sulfasalazine.

UC simulation in Balb/C mice was accompanied by an increase in the Krebs index by 7.3-10.9% (p=0.0033-0.0101), a decrease in LI by 28.3-30.0% (p=0.0009) and in LMRI – by 33.4-35.1% (p=0.0023-0.0054) on the 5th day of the experiment. The LI was significantly lower only in the control groups than in intact mice: by 21.1-22.4% (p=0.0009) on the 7th day of the experiment. In mice with chronic UC, all the studied indices were significantly different from similar values in intact rats on the 28th day. A decrease in the Krebs index was by 45.5% (p=0.0009), NMRI – by 46.2-49.2% (p=0.0009), and LMRI – by 30.2-30.8% (p=0.0039). The remaining indices, on the contrary, increased: LI – by 40.1-40.9% (p=0.0009), Bredeck index – by 3.87-3.92 times (p=0.0009), and LERI – by 2.41-2.43 times (p=0.0009).

Dalargin administration in UC mice was accompanied with a decrease in the Krebs index by 16.9% (p=0.0009), an increase in LI – by 21.8% (p=0.0009), in NMRI – by 34.5% (p=0.0054), and in LMRI – by 65.0% (p=0.0009) on the 5th day of the experiment. LERI was significantly higher only in mice of the UC+dalargin group compared to the control group by 96.7% (p=0.0357) on the 7th day. There were no significant differences between the compared groups in mice with chronic UC (p>0.05).

Sulfasalazine administration was accompanied by a decrease in the Krebs index by 14.8% (p=0.0028) and an increase in LMRI by 53.7% (p=0.0117) compared to control group No. 2. One parameter changed significantly only in mice of the UC+sulfasalazine group: LI was less by 7.5% (p=0.0157) on the 28th day. Comparing the effects of the drugs revealed that the Krebs index was higher in the UC+sulfasalazine group by 6.1% (p=0.0274), LI was less by 6.3% (p=0.0274), and LMRI was less by 4.5% (p=0.0357) on the 5th day of the experiment. LERI was less in mice with UC treated with dalargin by 5.0% (p=0.0117) on the 7th day. Krebs index in mice of the UC+dalargin group was less by 6.7% (p=0.0136), LI was higher by 8.1% (p=0.0087), LMRI was higher by 15.7% (p=0.0039), and LERI – by 3.8% (p=0.0039) in mice with chronic UC.

The results obtained confirm the literature data on the increased severity of acute UC from the 5th to 7th days of disease development and a significant decrease in DAI, the prevalence of mucosal ulcers and an increase in the colonic length on the 28th day (chronic UC) (Khomyakova et al. 2016), as well as a decrease in the amount of GC, HSA and neutral mucins in the colonic crypts, both in UC patients (Ordás et al. 2012) and in mice with experimental UC (Zolotova et al. 2016). GC secrete mucus components, primarily mucins, which form the basis of the protective barrier of the mucous membrane, preventing the penetration of pathogenic and commensal microflora, toxins from the lumen into the colonic wall and are important in the regulation of innate immunity (Ma et al. 2018). Disturbances in the colonic barrier and mucin layer destruction caused a significant permeability increase, bacteria penetration into the mucous membrane and submucosal layer, neutrophils, macrophages and lymphocytes activation with the subsequent development of immune inflammation (Davydova et al. 2022). The results manifest a decrease in the GC number, primarily at the bottom of the crypts, which seems extremely important, since it was previously shown that GC located at the bottom of the crypts produce the antimicrobial peptide WFDC2 in healthy people, and its secretion is impaired in UC patients (Parikh et al. 2019). This peptide manifests antiprotease activity by inhibiting the serine and cysteine proteases action. This effect of WFDC2 prevents the premature transformation of the inner layer of colon mucus into its outer layer. Destruction of the inner layer under the influence of proteases allows bacteria to penetrate into the colonic wall provoking the immune inflammation development (Ordás et al. 2012).

The results obtained confirm a significant decrease in the HSA mucins content in the colon mucosa in UC mice (Zolotova et al. 2016). It is generally accepted that HSA mucins are more effective than neutral mucins in preventing the destruction of the protective barrier of the colon mucosa by proteases (Pelaseyed et al. 2014). Previously, an increase in the neutral mucins content in the colon mucosa of UC mice was shown in (Zolotova et al. 2016). In our study, the number of neutral mucins decreased throughout the experiment. Multidirectional changes in neutral mucins content may be explained by the UC formation of varying severity in mice of different strains. These results confirm the literature data on an increase in the mucins content in the colon mucosa during the chronic UC development (Zolotova et al. 2016).

Dalargin administration at a dose of 100 mcg/kg subcutaneously once a day for 7 days from the beginning of UC simulation had a corrective effect on the disease development, which was manifested by a DAI decrease on the 7th day, the drop in pathological colonic shortening, a decrease in the ulcers and infiltrates prevalence in the colon mucosa on the 5th and 7th days. The results obtained allow us to conclude that the severity of acute UC is reduced in dalargin administration. The severity of the disease manifestations was significantly less in animals with chronic UC, but the dalargin corrective effect was shown even in the chronic process, which was manifested by a decrease in the ulcers and inflammatory infiltrates prevalence. The colon length in UC mice treated with dalargin on the 5th and 7th days was greater than in animals in the control group. A decrease in the mucosal ulcers prevalence on the 7th day and in the infiltrates in the LPM prevalence on the 5th and 28th days was observed in mice treated with dalargin. The study found that the dalargin administration in UC mice had a pronounced corrective effect on the disease manifestations: the amount of GC, HSA and neutral mucins increases. Thus, the results obtained indicate that dalargin had a therapeutic effect in experimental UC, while the pharmacological effect of dalargin is higher than that of sulfasalazine, which was manifested by a decrease in pathological colonic shortening in UC mice treated with dalargin on the 5th and 7th days, a decrease in the mucosal ulcers prevalence on the 7th day and the LPM infiltrates prevalence on the 5th and 28th days compared to the UC+sulfasalazine group.

It is known that dalargin is an analogue of leu-enkephalin and is capable to bind δ- and μ-OR (Bulgakov 2018). The mechanism of the dalargin therapeutic effect in colon inflammation is apparently explained by the μ-OR activation, since it was previously shown that their activation by the selective μ-ligand DAMGO had a corrective effect on the DSS-induced colitis development in mice (Anselmi et al. 2015). In particular, there was a decrease in DAI, myeloperoxidase activity, increased production of the anti-apoptotic factor Bcl-xl, a decrease in the pro-inflammatory cytokines and nuclear factor kB

concentration, which regulates the prostaglandins production (Anselmi et al. 2015). Researchers associate DAMGO effect with the peripheral μ-receptors activation, since DAMGO cannot penetrate the blood-brain barrier, and the preliminary injection of the peripheral μ-receptor antagonist CTAP eliminated its effect (Anselmi et al. 2015).

Further experiments were on elucidating the mechanisms of the therapeutic dalargin effect in murine UC. Previously, an increase in the pro-inflammatory IL content: IL-1β, IL-6, IL-17, was established in the plasma of UC patients and animals with experimental UC (Iwakura et al. 2011; Tatiya-Aphiradee et al. 2018; Schoultz et al. 2019; de Goulart et al. 2020; Rawat et al. 2020). An increase in the IL-17 concentration causes an increase in the permeability of the barrier of the colon mucosa, the leukocytes migration into its wall, as well as the production of other pro-inflammatory cytokines (IL-6), provoking the inflammation development (Iwakura et al. 2011; Lin et al. 2018). IL-1β and IL-6 disrupt, among other things, the mucus secretion by GC, reducing the protective mechanisms of the colon mucosa (Nishida et al. 2018; Wang et al. 2019). Previously, a direct relationship was shown between the UC severity and the IL-1β and IL-6 concentration in patients and laboratory animals (Saez-Lara et al. 2015).

An increase in the IL-10 content in plasma and in mononuclear cells of the colonic submucosal layer in UC patients was established (Braat et al. 2003). It is known that IL-10 is a main inhibitor of the Th1-induced immune response and the production of proinflammatory cytokines (TNFα, IL-1, IL-6), as well as an activator of regulatory T-cells (Treg), which are expected to be a promising target therapy for inflammatory bowel diseases (Katsannos and Papadakis 2017). The IL-4 role in the UC development is explained by the formation of the M2 population of macrophages suppressing inflammatory and stimulating reparative processes in the colon in UC, reducing the severity of clinical manifestations of the disease (Zhou et al. 2019). However, IL-4 content in UC changes contradictorily (Zhu et al. 2019).

The results obtained confirm the literature data on an increase in the content of IL-1β, IL-6, IL-10, IL-17 and a decrease in the IL-4 concentration in the colonic wall during the acute UC period (Zolotova et al. 2015;, Tatiya-Aphiradee et al. 2018; Schoultz et al. 2019; de Goulart et al. 2020; Jialing et al. 2020; Rawat et al. 2020). The formation of chronic UC in animals manifests itself either through the IL content normalization or even through their significant decrease in the wall of the medial colon (Zolotova et al. 2015). This is apparently explained by a change in the composition of the cell population in the colonic wall compared to the similar indices during the acute process and is characterized by an increase in B-lymphocytes (Gao et al. 2018).

Dalargin had a corrective effect on the balance of pro- and anti-inflammatory cytokines in the homogenate of the medial colon wall, manifested by a decrease in the concentration of IL-1β, IL-6, IL-17, as well as an increase in the IL-4 and IL-10 content. The mechanism of the immunomodulatory dalargin effect in colon inflammation is apparently explained by the activation of opioid μ-receptors. An increase in the expression of opioid μ-receptor mRNA was found during the acute period of disease development in both in UC patients (Phillippe et al. 2006) and in experimental UC in laboratory animals (Anselmi et al. 2015). Normal expression of μ-OR mRNA was observed in chronic UC colon inflammation (Phillippe et al. 2006; Anselmi et al. 2015).

μ-OR are previously shown on neutrophils, macrophages, T- and B-lymphocytes, dendritic, epithelial cells and colon neurons (Sternini et al. 2006; Lashgari et al. 2021), and their expression increases during the UC development (Valdez-Morales et al. 2013). The number of mononuclear cells containing μ-OR in the lamina propria of the mucous membrane increases in patients with UC (Phillippe et al. 2006). Increased β-endorphin and met-enkephalin formation manifesting high affinity for μ-OR, by immune cells in the colon mucosa was established both in experimental UC and in UC patients (Phillippe et al. 2006; Owczarek et al. 2011). These opioids manifest not only an analgesic but also an anti-inflammatory effect in experimental UC (Basso et al. 2018). According to the proposed hypothesis, enkephalins are autoregulators of T-lymphocyte activity and reduce their pro-inflammatory effect by inhibition the cytokines production (Basso et al. 2018).

Agonists of opioid μ-receptors have an inhibitory effect on the pro-inflammatory cytokines content in animals subjected to various models of experimental UC (Phillippe et al. 2006; Di Cello et al. 2018). Effect of the selective μ-OR agonist DAMGO on the DSS-induced colitis in mice decreased both the concentration of pro-inflammatory cytokines (IL-1β, IL-6, TNFα) and the clinical UC manifestations, including DAI (Anselmi et al. 2015). The researchers attribute DAMGO effect to the activation of peripheral μ-receptors.

Opioid δ receptors are also present in the colon, but predominantly on nerve plexus cells, and they regulate intestinal motility (Di Cello et al. 2018). The number of nerve fibers in the circular muscles containing δ-OR increases and their activation suppresses colon motility in DSS-induced colitis in mice (Di Cello et al. 2018).

Some researchers stress the IL-10 main role in the regulation of immune homeostasis in the colon (Neuman et al. 2019). The results obtained confirm an increase in the IL-10 concentration during the acute UC period (Wang et al. 2020). Previously, it was shown that IL-10 inhibits the secretion of nuclear factor kB and the proliferation of T-cells in patients with inflammatory bowel disease (Wang et al. 2020). An increase in the IL-10 content in the homogenate of the colon wall in mice with UC treated with dalargin is one of the mechanisms of the anti-inflammatory drug effect.

Possible informative markers of the development of inflammation in the colon wall are MMP (de Almeida et al. 2022). MMP activity is regulated by special proteins – TIMP. Currently, 4 TIMPs have been established: 1, 2, 3, 4, and their effect on various MMP is nonspecific (Maronek et al. 2021; de Almeida et al. 2022). MMP and TIMP expression increases during the development of inflammatory diseases such as rheumatoid arthritis, osteoarthritis, psoriasis, chronic obstructive pulmonary disease, and fetal growth retardation (Efremova 2022).

The MMP concentration in the colon is low under conditions of physiological rest, but it increases significantly in acute inflammation accompanied with the disruption of the barrier function of the colon mucosa due to the destruction of the intercellular substance, including mucins and claudins (Maronek et al. 2021). The concentration of various MMP increases in the colon both in experimental studies (Maronek et al. 2021) and in UC patients (Cabral-Pacheco et al. 2020). MMP-2 and MMP-9 are of particular

interest to researchers (Lin et al. 2018; Eiro et al. 2023). MMP-2 and MPP-9 are gelatinases regulating various parts of the UC pathogenesis (de Almeida et al. 2022). MMP-2 is involved in the remodeling of collagen structures and prevents the development of neutrophil infiltration and fibrosis in colon tissue (Maronek et al. 2021). MMP-9 stimulates the development of neutrophil infiltration during inflammation, disrupts reparative processes in the epithelium, increases the permeability of the vascular wall, and causes activation of cytokines (IL-1β, IL-8, transforming growth factor-β) in addition to participating in the remodeling of the extracellular matrix (Maronek et al. 2021). Some authors emphasize the key role of MMP-9 in the UC progression (Lin et al. 2018). An increase in the MMP-9 plasma concentration was also shown to correlate with the UC severity (Lin et al. 2018; Eiro et al. 2023). TIMP-2 manifests an inhibitory effect on the MMP-2, MMP-9 and MMP-14 activity (Cabral-Pacheco et al. 2020).

The results obtained confirm an increase in MMP-2, MMP-9 and TIMP-2 contents during the UC development, both in laboratory animals and in patients (Derkacz et al. 2021). However, the dynamics of the measured parameters concentration was different: if the contents of MMP-2 and TIMP-2 turned out to be maximal on the 5th day of the experiment, then the MMP-9 concentration was maximal on the 7th day. As shown previously, the experimental UC severity in laboratory mice increases from the 5th to 7th days (Derkacz et al. 2021). The data obtained confirm that the MMP-9 dynamics in UC is directly proportional to the disease severity, and changes in the MMP-2 and TIMP-2 concentrations are inversely proportional (Derkacz et al. 2021). The MMP and TIMP-2 contents are significantly reduced in murine chronic UC, and the MMP concentration does not differ significantly compared to that in intact mice. These are apparently explained by changes in the leukocyte population at the inflammatory focus and the predominance of B-lymphocytes (Luo et al. 2019). It was previously shown that the MMP-9 plasma concentration in patients with acute UC is significantly higher than in patients with remission or in healthy volunteers, which indicates the possibility of using the MMP-9 concentration as a marker of the inflammatory process activity in the colon and the treatment effectiveness (Fan et al. 2021; Shamsheya et al. 2021). TIMP concentration increases in inflammation, which is a protective mechanism (Shamsheya et al. 2021). However, as shown in most studies, increasing TIMP levels are usually not able to prevent MMP activation (Shamsheya et al. 2021).

The pharmacological dalargin effect on the MMP-2, MMP-9 and TIMP-2 contents in UC mice established in our study is apparently associated with the activation of opioid μ-receptors on mono- and polynuclear cells in the colonic wall, manifesting a decrease in their functional activity, reduction of MMP production by macrophages, lymphocytes and neutrophils. The dalargin binding to opioid δ receptors on nerve plexus cells decreases the peristalsis and perhaps reduces indirectly the colonic inflammation intensity.

The inhibitory effect of opioid receptor agonists on MMP-9 was established in (Ronsisvalle et al. 2019): the endogenous opioid μ-receptor agonist endomorphin-2 has an inhibitory effect on MMP-9, and the synthetic opioids MML617, MML717 and MML1017 inhibit the activity of MMP-2 and MMP-9.

A decrease in the activity of opioid κ-receptors, MMP-2 activity increase and the progression of a malignant tumor were found in patients with esophageal cancer (Huang et al. 2022).

The TGF-β and EGF involvement in the UC development has now been confirmed. TGF-β suppresses the development of the immune response to antigens of commensal microflora and is involved in the formation of immunological tolerance in the colon (Triantafillidis et al. 2020). TGF-β promotes reparative processes in the mucous colonic membrane in UC (Liu et al. 2021); however, its excess production can contribute to the fibrosis development in the colonic wall (Naghdalipour et al. 2022), manifesting the formation of strictures requiring surgical treatment. The functional TGF–β activity is impaired in animals with experimental UC and patients with inflammatory bowel disease (IBD), and its correction can be considered as a potential way to treat UC (Tatiya-Aphiradee et al. 2018).

EGF has a pronounced stimulating effect on the migration and proliferation of fibroblasts, which helps to restore the integrity of the colonic barrier. The cytokine suppresses the immune response to colonic microbiota antigens, reducing the severity of colon damage in UC. Activation of EGF receptors has a corrective effect on the UC development, indicating the possibility of using EGF as a means of treating UC (Lu et al. 2014).

The results obtained confirm an increase in the TGF-β concentration, both in laboratory animals with experimental colitis and in UC patients (Tatiya-Aphiradee et al. 2018; Zhu et al. 2019). It was previously shown that a significant increase in the TGF-β concentration activates the TGF-β-Smad signaling pathway and an increase in the activity of Smad7, accompanied with a decrease in the anti-inflammatory TGF-β effect (Garo et al. 2019). In addition, it was found that Smad7suppression inhibits the activity of epithelial myosin light chain kinase and causes a decrease in the permeability of the epithelial barrier of the colon mucosa (Bai et al. 2022).

An increase in the EGF content was established on the 7th and 28th days of the experimental UC development. The protective effect of an EGF analogue on the IBD development was previously shown (Zhou et al. 2022). Activation of EGF receptors on epithelial colon cells has a cytoprotective effect and also suppresses the production of nuclear factor kB, TNFα and interferon-γ by macrophages (Lu et al. 2014). There is evidence about the conjugation of the TGF-β and EGF effects through the pro-inflammatory signaling pathway of TGF-β-EGF receptor (El Mahdy et al. 2023). The researchers associate the different drugs effects, among other things, with inhibition of this mechanism.

Dalargin administration subcutaneously at a dose of 100 mcg/kg once a day for 7 days had a corrective effect on the TGF-β content in the medial colon homogenate in UC mice: the TGF-β concentration was significantly less on the 5th and 7th days, and it was higher on the 28th day compared to those in the control group. The EGF content increased throughout the experiment in dalargin administration.

Dalargin effect on EGF was significantly higher than the sulfasalazine action throughout the experiment. But there were no significant differences in the effects of dalargin and sulfasalazine on the TGF-β content.

Apparently, the pharmacological dalargin effect on the TGF-β and EGF concentrations is explained by its stimulating effect on opioid μ-receptors localized on macrophages, neutrophils, and lymphocytes of the colon wall.

A decrease in TGF-β activity in dalargin administration inhibits the Smad7 activity contributing to the anti-inflammatory dalargin effect. An increase in EGF activity in the group UC+dalargin enhances reparative processes in the colon correcting the intestinal barrier permeability.

Our results confirm the literature data on the active participation of neutrophils in the UC development (Muthas et al. 2017). As shown by multicenter studies, the determination of the PICaSSO Histological Remission Index [PHRI], based only on the neutrophils determination, is a simple and reliable index of UC remission and correlates with the severity of endoscopic changes in the colon wall (Parigi et al. 2023).

Dalargin had a corrective effect on the phagocytic activity of neutrophils, manifested by an increase in PF, FI and OFI on the 5th and 7th days of the experiment compared to those in the control group. It can be assumed that the corrective dalargin effect on the functional activity of neutrophils is realized through opioid μ-receptors on the neutrophils surface. Also the therapeutic drug effect is apparently associated with a decrease in the production of pro-inflammatory cytokines and an increase in the anti-inflammatory IL secretion.

Changes in leukocyte indices in UC indicate the participation of immune mechanisms in the UC pathogenesis. It is generally accepted that LI characterizes the relationship between the humoral and cellular components of the immune system (Supilnikov and Shabalin 2018). A decrease in this index in acute UC indicates the inclusion of cellular immune mechanisms during the IBD development. LMRI characterizes the relationship between the affector and effector parts of the immune response (Supilnikov and Shabalin 2018). A significant LMRI increase in UC mice treated with dalargin on the 5th day of the experiment confirms the drug stimulating effect on the affector immunity component, and a decrease in this index on the 7th day confirms the effector component activation. It was previously shown that NMRI characterizes the microphage and macrophage components of the innate immune system (Supilnikov and Shabalin 2018). Apparently, the change in hematological indices on the 5th day and, mainly, on the 7th day is due to the corrective dalargin effect on the cell activity in the macrophage-monocyte pool.

Lipid peroxidation (LPO) activation is shown in various inflammatory diseases, including UC. The dugs administration inhibiting ferroptosis in UC, for example, sodium butyrate, caused a decrease in the clinical and morphological manifestations of DSS-induced colitis in mice: colon shortening, DAI reduction, and reduction in ulcer areas (Chen et al. 2024). Moreover, the sodium butyrate administration helped to keep the colonic barrier integrity and suppressed the secretion of proteins that induce ferroptosis. On the contrary, the formation of ferroptosis inhibitors: nuclear erythroid factor-2 and glutathione peroxidase-4 – significantly increased (Chen et al. 2024). It has been established that the administration of traditional Chinese medicine that inhibits ferroptosis has a pronounced corrective effect in UC (Guo et al. 2024). The mechanism of this effect is explained by an increase in glutathione content and an increase in the glutathione peroxidase-4 activity.

The antioxidant dalargin effect in inflammatory diseases, in hypoxia and in ischemia-reperfusion has been confirmed in numerous studies (Bulgakov 2018). LPO inhibition provoked by dalargin is explained by a decrease in the free radicals formation and an increase in the activity of low molecular weight antioxidants and antioxidant enzymes, primarily superoxide dismutase (Tadzhibova et al. 2010). The dalargin stimulating effect on DNA synthesis in the gastric epithelium of rats is associated with the correction of nitric oxide metabolism, LPO inhibition and increased activity of antioxidant systems (Zivotova et al. 2007). The presented data allow concluding that inhibition of free radical oxidation is one of the mechanisms of the corrective drug effect in IBD. Activated macrophages and neutrophils are known to be the most important producers of free radicals during inflammation. As shown in our study, dalargin administration reduced the inflammatory cytokines concentration, which indicates a decrease in the macrophages activity. Thus, the dalargin antioxidant effect in experimental UC may also be explained by a decrease in the free radicals production by macrophages.

The endothelial dysfunction development in UC patients has been confirmed in numerous studies (Gravina et al. 2018; Di Nardo et al. 2024) and is associated with impaired production of inflammatory cytokines, growth factors, nitric oxide, endothelial cell adhesion molecules and procoagulants, as well as oxidative stress activation (Gravina et al. 2018). An increase in the formation of asymmetric dimethylarginine in IBD, which inhibits the NO synthase activity, was shown by Hosseinzadeh-Attar et al. (2020). The manifestations of endothelial dysfunction in IBD are frequent thrombosis and thromboembolism (Harindranath et al. 2023). Increased coagulation properties of plasma are associated with the action of proinflammatory cytokines, primarily IL-6 and TNFα. It was established that dalargin has a therapeutic effect on the endothelial dysfunction development in patients with severe concomitant trauma, manifesting in normalization of the content of stable metabolites of nitric oxide and the content of angiotensin-converting enzyme in plasma (Antonova et al. 2023). The researchers indicate that the endothelium protective effect of dalargin may be due to several mechanisms: 1) a decrease in the amount of oxidized low-density lipoproteins, the final metabolite of which is malondialdehyde; 2) GSK-3β phosphorylation, providing protection of endothelial cells from ischemia-reperfusion; 3) increased activity of the nuclear transcription factor Nrf2, a regulator of endothelial cell activity and antioxidant enzymes: superoxide dismutase, catalase, and glutathione peroxidase. Dalargin also prevented the destruction of the adhesive junction protein VE-cadherin and the tight junction of protein claudin in endothelial cells when the endothelial cell culture was exposed to the serum of patients with septic shock (Grebenchikov et al. 2018). Thus, the endothelial protective effect of dalargin may be the mechanisms of its therapeutic effect in experimental UC.

The results obtained allow concluding that dalargin has a therapeutic effect in experimental UC. It has been shown that drug administration at a dose of 100 mcg/kg subcutaneously once a day for 7 days to mice with experimental UC caused a DAI decrease, a decrease in pathological colon shortening, a decrease in the ulcers and inflammatory infiltrates prevalence, and an increase in the GC, HSA and neutral mucins amounts. The corrective effect

of dalargin was higher than that of sulfasalazine, which is often administered in the UC treatment in patients and experimental colitis. The mechanisms of the therapeutic effect of dalargin in UC include: 1) a decrease in the pro-inflammatory cytokines content: IL-1β, IL-6, IL-17, an increase in the IL-10 and IL-4 concentrations in acute experimental UC; 2) a decrease in the contents of MMP-2, MMP-9 and TIMP-2, which was shown in our study in animals with experimental UC; 3) a decrease in the TGF-β content and an increase in the EGF concentration, which indicate a decrease in inflammatory activity and acceleration of reparative processes in experimental UC; 4) suppression of the pathologically increased activity of peripheral blood neutrophils in experimental UC; 5) antioxidant effect and corrective effect on vascular damage.

The data obtained open up prospects for preclinical and clinical studies of the dalargin effects in UC. The results of the study make it possible to recommend the dalargin administration as a means for correcting UC or as a component of combination therapy to treat the disease.

Conclusion

1. Dalargin, administered subcutaneously at a dose of 100 mcg per kg body weight once a day for 7 days, had a corrective effect on the experimental UC development in Balb/C mice, which was manifested by a decrease in pathological colon shortening by 31.2% and 23.5%, in the ulcers prevalence – by 46.5% and 59.8%, in the infiltrates prevalence – by 25.4% and 44.2% on the 5th and 7th days of the experiment, respectively, and a decrease in DAI by 28.6% on the 7th day, compared to the control group. The drug pharmacological effect was higher than that of sulfasalazine.

2. Dalargin increased the production of HSA by 50.0% and 54.8% and of neutral mucins by 6.2% and 7.9% on the 5th and 7th days of the experiment, respectively, and the number of GC in UC mice on the 5th and 7th days of the experiment was higher by 3.6% and 19.3%, respectively, in mice with DSS-induced UC compared to that in control animals. The dalargin effect was significantly higher than that of sulfasalazine.

3. The corrective effect of dalargin on the chronic UC (the 28th day of the experiment) was manifested by a decrease in the ulcers and infiltrates prevalence by 9.1% and 46.7%, respectively, and the dalargin effect on the infiltrates prevalence infiltrates was more pronounced compared to that of sulfasalazine. The dalargin administration did not affect the GC amount, the HSA and neutral mucins content in chronic experimental UC.

4. The dalargin administration had a corrective effect on the pro- and anti-inflammatory cytokines content in the medial colon homogenate of mice with experimental UC, manifested by a decrease in the IL-1β concentration by 35.8% (the 5th day) and by 41.2% (the 7th day), IL-6 – by 55.6% (the 5th day), IL-17 – by 42.1% (the 5th day), by 48.8% (the 7th day), and by 45.5% (the 28th day), as well as an increase in the IL-10 content by 2.19 times (the 5th day), by 59.6% (the 7th day), and by 6.79 times (the 28th day), and in the IL-4 content – by 29.1% (the 5th day) and by 50.0 % (the 7th day) compared to those in the control group.

5. The dalargin decreased significantly the MMP-2 concentration by 6.3 times (the 5th day) and by 3.9 times (the 7th day), the MMP-9 concentration – by 4.1 times (the 5th day) and by 5.6 times (the 7th day), the TIMP-2 concentration – by 46.7% (the 5th day), by 35.4% (the 7th day), and by 3.7 times (the 28th day) compared to those in the control group.

6. Dalargin had a corrective effect on the growth factors content in mice with experimental UC, manifested by a decrease in the TGF-β content on the 5th and 7th days of the experiment compared to those in the control group by 49.4% and 45.8%, respectively, an increase in the EGF concentration on the 5th day – by 91.7%, on the 7th day – by 2.4 times, and on the 28th day – by 2.9 times. The TGF-β concentration was higher than that in the control group by 40.4% in chronic colitis.

7. Dalargin had a modulating effect on the phagocytic activity of neutrophils, which is confirmed by a decrease in PI by 32.4%, PN – by 16.7%, OPI – by 39.8% on the 5th day and by 22.4%, 17.6%, 36.6%, respectively, on the 7th day compared to the control group in mice with experimental colitis. Drug did not have a significant effect on the neutrophils phagocytic activity on the 28th day.

8. Dalargin administration decreased the Krebs index by 16.9%, increased LI by 21.8%, NMRI – by 34.5%, and LMRI – by 65.0% on the 5th day of the experiment. LERI was significantly higher in mice of the UC + dalargin group only compared to that in the control group by 96.7% on the 7th day. There were no significant differences between the compared groups on the 28th day (chronic colitis).

Conflict of interest

The authors declare the absence of a conflict of interests.

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Data availability

All of the data that support the findings of this study are available in the main text.

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