УДК 616.348-002.44-021.6-085.243:616.345-018.25-018.1
https://doi.org/10.20538/1682-0363-2024-3-59-65

Effect of dalargin on the content of goblet cells and mucins in the colonic mucosa in experimental ulcerative colitis

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ABSTRACT

Aim. To investigate the protective effect of dalargin on the content of goblet cells and mucins in the colonic mucosa in a mouse model of ulcerative colitis.

Materials and methods. Ulcerative colitis was simulated in Balb/C mice by replacing drinking water with 5% sodium dextran sulfate in boiled water for 5 days. Dalargin was administered subcutaneously in a volume of 0.1 ml at a dose of 100 μg / kg of body weight once a day for 7 days from the beginning of ulcerative colitis simulation. Sulfasalazine as a reference-listed drug was administered intragastrically at a dose of 200 mg / kg once a day for 7 days. The mice were sacrificed on day 5, 7, and 28. The sections of the distal colon were prepared and stained with hematoxylin and eosin, alcian blue (pH = 1.0) according to Mowry or by PAS reaction. In the sections, the number of goblet cells and acid and neutral mucins was determined.

Results. In the mouse model of ulcerative colitis, the number of goblet cells (mainly at the bottom of the crypts), acid and neutral mucins decreased. Dalargin administration increased the number of goblet cells and the content of acid and neutral mucins in the colonic mucosa more effectively than sulfasalazine.

Conclusion. Dalargin has a protective effect in ulcerative colitis.

Keywords: ulcerative colitis, dalargin, sulfasalazine, goblet cells, mucins

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

Source of financing. The authors state that they received no funding for the study.

Conformity with the principles of ethics. The study was approved by the Ethics Committee Kursk State Medical University (Protocol No. 1 of 03.04.2023).

For citation: Liashev A.Yu., Mal G.S., Solin A.V. Effect of dalargin on the content of goblet cells and mucins in the colonic mucosa in experimental ulcerative colitis. *Bulletin of Siberian Medicine.* 2024;23(3):59–65. https://doi.org/10.20538/1682-0363-2024-3-59-65.

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Влияние даларгина на содержание бокаловидных клеток и муцинов в слизистой оболочке толстого кишечника при экспериментальном язвенном колите

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РЕЗЮМЕ

Цель – изучение протективного эффекта даларгина на содержание бокаловидных клеток и муцинов в слизистой оболочке толстого кишечника у мышей с экспериментальным язвенным колитом.

Материалы и методы. Язвенный колит моделировали у мышей линии Balb/C заменой в течение 5 сут питьевой воды 5%-м раствором декстрана сульфата натрия в кипяченой воде. Даларгин вводили подкожно в объеме 0,1 мл в дозе 100 мкг/кг массы тела 1 раз/сут в течение 7 сут с начала моделирования язвенного колита. Препарат сравнения сульфасалазин вводили в желудок в дозе 200 мг/кг 1 раз/сут в течение 7 сут. На 5, 7 и 28-е сут мышей выводили из эксперимента. На депарафинированных, окрашенных гематоксилином и эозином, альциановым синим (pH = 1,0) по Моури или реактивом Шиффа срезах дистального отдела ободочной кишки определяли количество бокаловидных клеток, содержание кислых и нейтральных муцинов.

Результаты. При модели язвенного колита в дистальном отделе ободочной кишки мышей снижается количество бокаловидных клеток (преимущественно в основании крипт), кислых и нейтральных муцинов. Даларгин эффективнее сульфасалазина увеличивает количество бокаловидных клеток, содержание кислых и нейтральных муцинов.

Заключение. Даларгин оказывает протективное влияние при экспериментальном язвенном колите.

Ключевые слова: модель язвенного колита, даларгин, сульфасалазин, бокаловидные клетки, муцины

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Исследование одобрено региональным этическим комитетом КГМУ (протокол № 1 от 03.04.2023).

Для цитирования: Ляшев А.Ю., Маль Г.С., Солин А.В. Влияние даларгина на содержание бокаловидных клеток и муцинов в слизистой оболочке толстого кишечника при экспериментальном язвенном колите. *Бюллетень сибирской медицины.* 2024;23(3):59–65. https://doi.org/10.20538/1682-0363-2024-3-59-65.

INTRODUCTION

Goblet cells (GCs), along with absorptive colonocytes and enteroendocrine cells, are the main cell populations in the colonic mucosa [1]. GCs secrete mucus components, primarily mucins, which form the basis for the protective barrier of the mucous membrane. Mucins prevent the penetration of pathogenic and commensal microflora and toxins from the lumen into the wall of the colon and play an important role in the regulation of innate immunity [2]. There are two types of mucins: secretory and membrane-associated. Secretory mucins form an inner layer, which is impermeable to bacteria and high-molecular substances, while membrane-associated mucins form the glycocalyx [2]. Colonic barrier dysfunction and, primarily, damage to the mucin layer lead to a significant increase in permeability, penetration of bacteria into the mucous

membrane and submucosal layer, and activation of neutrophils [3], then macrophages and lymphocytes with the subsequent development of inflammation, which underlies the pathogenesis of ulcerative colitis (UC) [4].

UC is a chronic, relapsing disease of the colon. It develops in people aged 20–40 years, significantly worsens the quality of life, and often leads to disability [5]. Despite a significant number of investigations devoted to various UC aspects, its etiology and pathogenesis remain poorly understood, which results in low effectiveness of existing methods of its treatment [5]. In this regard, studying the effect of drugs exerting antioxidant, anti-inflammatory, and immunomodulatory effects on UC development is relevant.

Dalargin is one of these drugs, proposed for the treatment of peptic ulcers. Dalargin is also included in the pharmacotherapy of acute pancreatitis [6]. In this regard, it is of interest to study the effect of dalargin on the content of colonic mucins in a UC model.

The aim of the study was to investigate the protective effect of dalargin on the content of GCs and mucins in the colonic mucosa in a mouse model of UC.

MATERIALS AND METHODS

The experiments included 102 male Balb/C mice weighing 21–23 g, purchased from the Stolbovaya division of the Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency. The study was performed in compliance with the provisions of the Declaration of Helsinki proposed by the World Medical Association on Humane Treatment of Laboratory Animals (2000), the European Community Directive (86/609EC), and the Rules of Good Laboratory Practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 199n of 01.04.2016). Conducting experiments on the topic of the thesis research was approved by the regional Ethics Committee (Protocol No. 1 of 03.04.2023).

UC was modeled in mice by replacing drinking water with a 5% solution of dextran sulfate sodium (DSS) (Mr = 40,000, PanReac-AppliChem, Germany) in boiled water for 5 days [7]. The animals were euthanized when acute UC developed on day 5 and 7 and chronic UC developed on day 28.

Dalargin (Microgen, Russian Federation) was dissolved in normal saline and administered subcutaneously in a volume of 0.1 ml at a dose of 100 μg / kg of body weight once a day for 7 days from the beginning of UC modeling. According to the literature data, dalargin exhibits high pharmacological activity at the indicated dose [6]. Sulfasalazine (KRKA, Slovenia) was administered as a reference-listed drug into the stomach in the form of a suspension dissolved in normal saline in a volume of 0.3 ml at a dose of 200 μg / kg of body weight for 7 days from the beginning of UC modeling [8].

All animals were divided into the following experimental groups: 1) naïve mice, $n = 6$; 2) control group 1 (UC modeling + subcutaneous injection of normal saline), $n = 24$; 3) control group 2 (UC modeling + intragastric injection of normal saline), $n = 24$; 4) experimental group 1 (UC modeling + dalargin administration), $n = 24$; 5) experimental group 2 (UC modeling + sulfasalazine administration), $n = 24$.

The mice were euthanized by cervical dislocation under chloral hydrate anesthesia on days 5, 7, and 28. The distal colon was placed in neutral buffered 10% formaldehyde solution. Deparaffinized 5–6-μm slices of the colon were stained with hematoxylin and eosin, alcian blue (pH = 1.0) according to Mowry to detect highly sulfated acid mucins (AM) or by PAS reaction to detect neutral mucins (NM). Light microscopy was performed on the Nicon Eclipse Ni microscope using the NIS Elements AR software. The stained slices were scanned on the Hamamatsu NanoZoomer-SQ digital slide scanner (Japan). The resulting digital images were analyzed in the QuPath program [9] using the color deconvolution technique [10].

To assess AM and NM content in the colonic mucosa, histologic preparations after treatment with iodine acid were digitized. Areas with longitudinally oriented crypts without ulcers and erosions, but with pronounced signs of inflammation were identified in the digital images obtained. The number of GCs per 1 crypt was determined on slices stained with alcian blue. The content of AM and NM was assessed by the intensity of GC staining with alcian blue and PAS reaction after treatment with iodine acid, respectively. The coloring intensity was calculated as the average value of the decimal logarithm of the background brightness / image object point brightness ratio [11]. The intensity of histochemical

reactions varied significantly even in the control groups, which is associated with differences in slice thickness, fixation time, and staining. The intensity of GC staining was normalized by the intensity of staining of adjacent areas of connective tissue to level out these differences.

All samples were checked for normality of distribution using the Shapiro – Wilk test and for homogeneity of variance using the Levene's test. The Mann – Whitney *U*-test was used to determine the significance of differences. Nonparametric statistics methods were used due to small sample sizes, different distribution patterns, and heterogeneity of variance when comparing the samples. The results were described as the median and the interquartile range Me (Q_1; Q_3). The null hypothesis was rejected at the level of statistical significance $p \leq 0.05$. Statistical analysis of the results was carried out using the Statistica 10.0 software package.

RESULTS

The number of GCs decreased by 32.7–33.2% on day 5, by 47.1–47.6% on day 7, and by 15.4–15.9% on day 28 of the experiment ($p = 0.0024$) in both control groups of mice with experimental UC (Table). The number of GCs mainly decreased at the bottom of the crypts (Figure). GCs expanded, and their area increased. In mice with chronic UC (day 28), the number of GCs was 25.7–61.5% higher than in the animals with acute UC (day 5–7) ($p = 0.0009$). The content of AM in the colonic mucosa decreased by 3.43–3.75 times and 3.64–3.87 times and the content of NM declined by 36.3% and 38.2–39.3% on day 5 and day 7 of the experiment, respectively ($p = 0.0024$), compared to naïve animals. The content of mucins in mice with chronic UC was higher than in mice with acute UC, but was lower than in the naïve group (AM – by 42.5–43.3%, NM – by 27.5–29.4%) ($p = 0.0024$)).

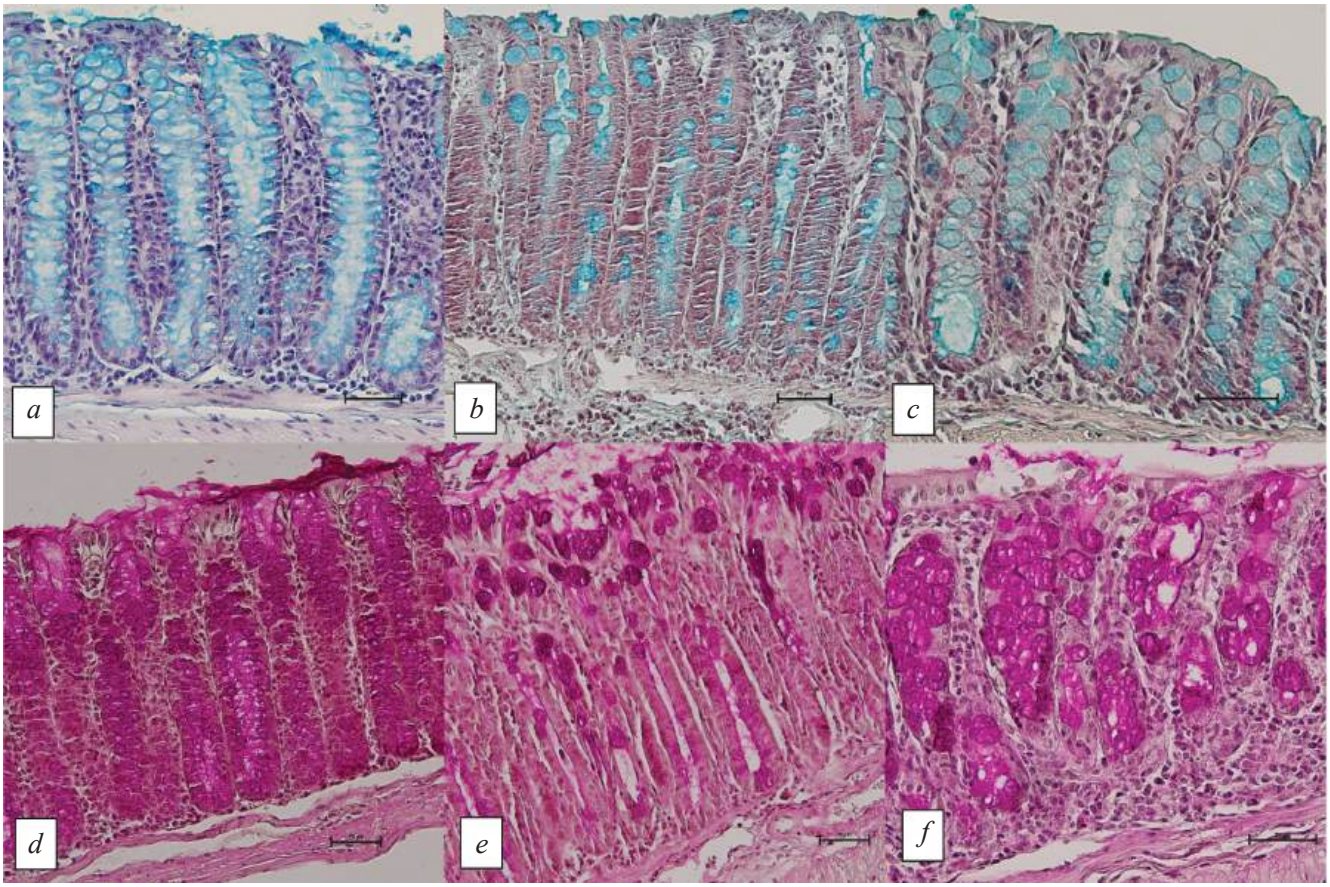

Figure. Goblet cells in the colon of male Balb/C mice: *a–c* – staining with hematoxylin and eosin + alcian blue, *d–f* – staining with hematoxylin and eosin + PAS reaction. *a*, *d* – control group, *b*, *e* – model of acute UC *c*, *f* – model of chronic UC. Bar – 50 μm

Dalargin administration was accompanied by an increase in the number of GCs in mice with the experimental UC by 3.6% (p = 0.0011) on day 5 of the experiment and by 9.3% (p = 0.0009) on day 7 compared to the control animals. Dalargin had no effect on the number of GCs in the colonic crypts of mice with chronic experimental UC. It increased the level of AM by 50.0% (p = 0.0239) on day 5 and by 54.8% (p = 0.0136) on day 7 of the experiment and increased the NM content by 6.2% (p = 0.0136) and 7.9% (p = 0.0009) on day 5 and day 7 of the experiment, respectively.

Table

The effect of dalargin and sulfasalazine on the number of goblet cells in crypts and the content of acid and neutral mucins in the colon of mice with experimental ulcerative colitis, *Me* [Q_1; Q_3]

No.	Experimental group	Duration of the experiment, day	Number of goblet cells, *n*	lg10 of the content of neutral mucins	lg10 of the content of highly sulfated acid mucins
Intact animals			20.8 [20.7; 20.9]	1.02 [1.00; 1.09]	1.20 [1.12; 1.35]
2.	Control group 1 (experimental UC + subcutaneous injection of normal saline)	5	14.0 [13.9; 14.1]^x p = 0.0024	0.65 [0.65; 0.66]^x p = 0.0024	0.32 [0.27; 0.43]^x p = 0.0024
		7	10.9 [10.7; 11.0]^x p = 0.0.0024	0.63 [0.63; 0.64]^x p = 0.0024	0.31 [0.28; 0.44]^x p = 0.0024
		28	17.6 [17.3;17.9]^x p = 0.0.0024	0.72 [0.72; 0.73]^x p = 0.0024	0.68 [0.58; 0.80]^x p = 0.0024
3.	Control group 2 (experimental UC + intragastric injection of normal saline)	5	13.9 [13.7; 14.3]^x p = 0.0024	0.65 [0.64; 0.66]^x p = 0.0024	0.35 [0.29; 0.43] ^{x} p = 0.0024; p = 0.0023
		7	11.0 [10.8; 11.2]^x p = 0.0024	0.64 [0.63; 0.64]^x p = 0.0024	0.33 [0.28; 0.37]^x p = 0.0024
		28	17.5 [17.4; 17.7]^x p = 0.0024	0.74 [0.74; 0.75]^x p = 0.0024	0.69 [0.54; 0.84]^x p = 0.0024
4.	Experimental UC + dalargin at a dose of 100 μg / kg subcutaneously	5	14.5 [14.3; 14.6]* p = 0.0011; ^1p = 0.3720	0.69 [0.68; 0.70]* p = 0.0136; ^1p = 0.2076;	0.48 [0.38; 0.56]* p = 0.0239; ^1p = 1.00
		7	13.0 [12.9; 13.2]*^{1} p = 0.0009; ^1p = 0.0009	0.68 [0.66; 0.71]*^{1} p = 0.0019; ^1p = 0.0019	0.48 [0.44; 0.60]* p = 0.0136; ^1p = 0.5635
		28	18.0 [17.7; 18.6] p = 0.0587; ^1p = 0.7527	0.78 [0.76; 0.80]^1 p = 0.0520; ^1p = 0.0023;	0.75 [0.68; 0.84] p = 0.3184; ^1p = 0.1722
5.	Experimental UC + sulfasalazine at a dose of 200 μg / kg intragastrically	5	14.3 [14.1; 14.5]* p = 0.0011	0.67 [0.66; 0.70]* p = 0.0101;	0.45 [0.38;0.55] p = 0.0831
		7	12.1 [11.9; 12.3]* p = 0.0009	0.63 [0.62; 0.64] p = 0.4623	0.45 [0.39;0.59]* p = 0.0209
		28	18.0 [17.7; 18.1] p = 0.0587	0.75 [0.74; 0.76]* p = 0.0136	0.65 [0.63; 0.75] p = 0.8337

$p < 0.05$ compared to the parameters in the: ^{x} – intact group; * – control group; ^{1} – animals treated with sulfasalazine.

The number of GCs increased by 2.9% (p = 0.0011) on day 5 and by 10.0% (p = 0.0009) on day 7 of the experiment, when sulfasalazine was administered at a dose of 200 μg / kg to mice with experimental UC, compared to the control group 2. The AM content did not change throughout the experiment, the NM content increased by 3.1% (p = 0.0101) on day 5 and by 3.1% (p = 0.0136) on day 28 of the experiment, compared to the control group 2.

Dalargin increased the number of GCs by 7.4% (p = 0.0009) on day 7 compared to the value during sulfasalazine administration. The NM content increased by 7.9% (p = 0.0019) on day 7 and by 8.3% (p = 0.0023) on day 28 only in the experiment with dalargin administration.

DISCUSSION

The results of the study confirm the literature data on a decrease in the number of GCs and the content of AM and NM in the colonic crypts both in patients with UC [12] and in a mouse model of UC [11]. The work shows a decrease in the number of GCs mainly at the bottom of the crypts. It is known that GCs located at the bottom of crypts produce the antimicrobial peptide WFDC2 in healthy people, while its secretion is impaired in patients with UC [12]. This peptide inhibits serine and cysteine proteases; therefore, it prevents premature transformation of the inner layer of the colonic mucosa into the outer layer. The outer layer is permeable to bacteria coming from the colonic lumen and serves as a growth medium for the commensal microflora; the inner layer is

impermeable to bacteria and ensures the maintenance of the colonic wall homeostasis [13]. Destruction of the inner layer under the influence of proteases promotes penetration of bacteria into the colonic wall and development of inflammation in UC [4].

Highly sulphated AM and other acid mucins are more effective than NM in preventing the destruction of the protective colonic barrier by proteases [13]. The NM content increased in the colonic mucosa of mice with experimental UC [11]. In our study, the number of NM decreased throughout the experiment. Diverse changes in the NM content are due to the formation of experimental UC of varying severity in mice of different strains. The mucin content increases in the colonic mucosa during the transition from acute to chronic UC.

Dalargin has a protective effect in mice with experimental UC and is more effective than sulfasalazine, as it increases the number of GCs and the content of AM and NM. Dalargin, as an analogue of leu-enkephalin, activates opioid δ- and μ-receptors [6]. The mechanism of the therapeutic effect of dalargin in colonic inflammation is apparently explained by the activation of opioid μ-receptors, since their activation by the selective ligand DAMGO (H-Tyr-D-Ala-Gly-N-MePhe-Gly-ol) has a protective effect on DSS-induced UC development in mice [14]. Apparently, like DAMGO, dalargin reduces disease severity, decreases myeloperoxidase activity and the concentration of proinflammatory cytokines, prostaglandins, and nuclear factor kB, and increases the production of the antiapoptotic factor Bcl-xL [10]. The authors associate this effect with the activation of peripheral μ-receptors, since DAMGO is not able to penetrate the blood – brain barrier, and the peripheral μ-receptor antagonist CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Pen-Thr-NH_2) eliminates its effect [10].

Dalargin has antioxidant and immunomodulatory effects [6]. It inhibits the activity of mononuclear cells in their pathological activation [15] and, thus, reduces the severity of inflammation. Suppression of lipid peroxidation not only weakens alterative changes at the inflammation site, but also prevents an increase in the colonic barrier permeability and penetration of pathogenic microbes into the colon.

CONCLUSION

The protective effect of dalargin on the UC development in Balb/C mice was established: an increase in the number of GCs and the content of AM and NM in the colonic mucosa was registered. The effect of dalargin is more pronounced than that of sulfasalazine. The data obtained allow to consider dalargin as a component of an effective combination drug therapy for UC.

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Received 06.07.2023;
approved after peer review 14.09.2023;
accepted 25.01.2024