КЛИНИКО-ИММУНОЛОГИЧЕСКИЙ АНАЛИЗ ЭФФЕКТИВНОСТИ ЛОКАЛЬНОГО ПРИМЕНЕНИЯ ВИТАМИНА D_3 ПРИ ЭКСПЕРИМЕНТАЛЬНОМ КОЛИТЕ

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Резюме. Патогенез воспалительных заболеваний кишечника до конца не изучен, а используемые средства терапии имеют побочные эффекты, ограничивающие их применение.

Целью данного исследования является проведение клинико-иммунологического анализа эффективности применения витамина D_3 в составе оригинальных ректальных суппозиториев при экспериментальном колите (ЭК).

ЭК моделировали оксазолоном. Оригинальные суппозитории с витамином D_3 в 3-й группе, и 5-АСК в 4-й группе применяли *per rectum*. Клинику оценивали по шкале Disease activity index. В очаге повреждения толстой кишки определяли экспрессию MPO и TNFα, содержание нейтрофилов, лимфоцитов, эозинофилов, гистиоцитов, плазмоцитов, фибробластов, язвенный дефект, tissue damage index. Исследование проводили на 2-е, 4-е и 6-е сутки.

При ЭК на все сутки повышается DAI, в очаге повреждения увеличивается MPO и TNFα, фиксируется язвенный дефект, нейтрофильно-лимфоцитарная инфильтрация, увеличивается TDI. При сравнении морфометрических параметров зоны альтерации при ЭК в условиях применения витамина D_3 в отличие от применения 5-АСК выявлено на 2-е сутки снижение количества лимфоцитов, увеличение фибробластов, на 4-е сутки уменьшение количества плазмоцитов и увеличение фибробластов, на 6-е сутки увеличение количества гистиоцитов и фибробластов. Диаметр язвенного дефекта и индекс TDI не имеют значимых различий между сравниваемыми группами. При сравнении эффективности применения витамина D_3 в отличие от применения с 5-АСК содержание MPO выше на 6-е сутки, содержание TNFα – на 4-е сутки.

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При ЭК эффекты применения ректальных суппозиториев с витамином D_3 на клинические признаки, размер язвенного дефекта, содержание MPO и TNFα в очаге повреждения сопоставимы с эффектами от применения ректальных суппозиториев с 50 мг 5-АСК; более выражены в отношении динамики клеточного состава очага повреждения толстой кишки.

Ключевые слова: экспериментальный колит, витамин D_3, индекс активности болезни, MPO, TNFα, 5-аминосалициловая кислота

CLINICAL AND IMMUNOLOGICAL ANALYSIS OF THE EFFECTIVENESS OF LOCAL APPLICATION OF VITAMIN D_3 IN EXPERIMENTAL COLITIS

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Abstract. The pathogenesis of inflammatory bowel diseases has not been fully studied, and the therapies used have side effects that limit their use.

The purpose of this study is to conduct a clinical and immunological analysis of the effectiveness of vitamin D_3 in the original rectal suppositories in experimental colitis (EC).

EC was modeled with oxazolone. Original suppositories with vitamin D_3 in group 3 and 5-ASA in group 4 were used *per rectum*. The clinic was evaluated on the Disease activity index scale. The expression of MPO and TNFα, the content of neutrophils, lymphocytes, eosinophils, histiocytes, plasmocytes, fibroblasts, ulcerative defect, tissue damage index were determined in the focus of colon injury. The study was carried out on days 2, 4 and 6.

With EC, DAI increases for the entire day, MPO and TNFα increase in the lesion, ulcerative defect is fixed, neutrophil-lymphocytic infiltration increases, and TDI increases. When comparing the morphometric parameters of the alteration zone in EC under the conditions of vitamin D_3 use, in contrast to the use of 5-ASA, a decrease in the number of lymphocytes, an increase in fibroblasts was revealed on day 2, a decrease in the number of plasmocytes and an increase in fibroblasts on day 4, an increase in the number of histiocytes and fibroblasts on day 6. The diameter of the ulcerative defect and the TDI index have no significant differences between the compared groups. When comparing the effectiveness of vitamin D_3, in contrast to the use of 5-ASA, the MPO content is higher on day 6; the TNFα content is higher on day 4.

In EC, the effects of using rectal suppositories with vitamin D_3 on clinical signs, the size of the ulcerative defect, the content of MPO and TNFα in the lesion are comparable to the effects of using rectal suppositories with 50 mg of 5-ASA; more pronounced with respect to the dynamics of the cellular composition of the lesion of the colon.

Keywords: experimental colitis, vitamin D_3, disease activity index, MPO, TNFα, 5-aminosalicylic acid

Introduction

Inflammatory bowel disease (IBD) is a long–term, chronic, inflammatory-destructive and progressive lesion of the gastrointestinal tract that occurs under the influence of trigger factors on a genetically predisposed organism, which requires constant therapy during exacerbation and maintenance therapy in remission.

The urgency of the problem is caused by the defeat of IBD of young, able-bodied people (the first age peak is 20-30 years old), as well as people of pre-retirement age (the second age peak is 50-60 years old) and the associated temporary or complete disability [5]. In recent years, there has been a tendency in a number of countries to increase the incidence of IBD in childhood and among people over 65 years of age [7]. The severity of IBD is determined by severe complications: intestinal (toxic megacolon, massive bleeding, perforation of the intestinal wall, colorectal cancer) and extra-intestinal (anemia, arthritis, sacroiliitis, hepatitis, cirrhosis of the liver, gangrenous

pyoderma, iritis, uveitis, episcleritis, thrombosis, etc.) [5].

In the pathogenesis of IBD, both Th2-dependent reactions involving IgM, IgG, and Th1-dependent reactions with increased production of IL-8, TNFα, etc. are important in the destruction of the intestinal wall. cytokines, activation of chemotaxis, absorption, killing activity of neutrophils, monocytes/macrophages, production of reactive oxygen species (ROS) and nitrogen. These changes at the morphological level lead to damage to the distal parts of the colon, destruction of intestinal crypts, hyperplasia of goblet cells, ulceration in the mucous membrane, fibrosis, which is clinically manifested by tenesmus, changes in stool consistency, admixture of blood in feces, body weight deficiency, and other symptoms, including intestinal and extra-intestinal complications [6].

IBD requires constant pharmacocorrection and lifelong administration of medications to maintain remission, many of which (derivatives of 5-aminosalicylic acid, glucocorticosteroids, immunosuppressors, genetically engineered biological drugs) have severe side effects, which reduces compliance in patients, leads to intolerance and ineffectiveness of therapy in at least 30% of patients [14]. Vitamin D_3, which has pleiotropic properties, including antioxidant, anti-inflammatory, immunomodulatory, etc., is of particular interest as a new therapeutic agent [13]. The use of vitamin D_3 in multiple sclerosis and psoriasis limits the severity of the inflammatory process and clinical manifestations due to an increase in IL-10 production and the amount of Treg in the blood, a change in the Th1/Th2 balance towards a Th2-dependent immune response [6]. In rheumatoid arthritis, vitamin D_3 inhibits Th17 activity and IL-17 production [1].

These facts are a prerequisite for the use of vitamin D_3 in IBD [4]. At the moment, there are no dosage forms with vitamin D_3 in the Russian Federation for local use *per rectum*, taking into account the impact on the focus of inflammation and the damaged area of the colon in IBD. We have developed original rectal suppositories with vitamin D_3 based on a 10% aqueous solution of vitamin D_3 (Patent 20.05.2019). We have previously demonstrated that vitamin D_3 in the original rectal suppositories in experimental colitis (EC) has a local antioxidant effect by limiting the formation of POL and OMB products; a systemic immunotropic effect by reducing the number of neutrophils, restoring the absorption and HCT-reducing activity of neutrophils, reducing the number of lymphocytes, including $CD3^+$ and $CD45RA^+$, reducing the concentration of IgM, IgG, IL-6 and IL-8 [8]. We believe that the systemic immunotropic effects of vitamin D_3 in the composition of the original rectal suppositories in EC are due to its local protective effect in the focus of damage to the colon.

The aim of the study was a clinical and immunological analysis of the effectiveness of vitamin D_3 in the original rectal suppositories in experimental colitis.

Materials and methods

The study was performed on mature male rats weighing 220-230 g of the Wistar line obtained from the federal state budgetary institution "Nursery of Laboratory Animals "Rappolovo" (SIC "Kurchatov Institute" – PLZH "Rappolovo"), under supervision in the experimental biological clinic of the Federal State Educational Institution of the Ministry of Health of the Russian Federation in compliance with the rules of good laboratory practice (order of the Ministry of Health of the Russian Federation No. 199n of 01.04.2016), Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, with free access to food and water, on a standard diet. The organization of the study was approved by the Ethics Committee of the South Ural State Medical University, Protocol No. 11 of 27.12.2017, Protocol No. 3 of 14.03.2022.

Using simple randomization, 70 animals were divided into groups: I-I (n = 7) – intact control, II-I (n = 21) – animals with experimental colitis (EC), III-I (n = 21) – animals with EC under conditions of rectal administration of vitamin D_3 every 12 hours before withdrawal from the experiment on day 6, IV (n = 21) – animals with EC, under conditions of rectal use of 5-aminosalicylic acid (5-ASA) every 12 hours before withdrawal from the experiment on day 6.

EC was modeled by two-stage application of a 3% alcohol solution of oxazolone (4-ethoxymethylene-2-phenyl-2-oxazoline-5-oh). At the first stage, cutaneous sensitization was carried out by applying 675 μl/kg to the interscapular area, at the second – rectal injection of 675 μl/kg into the colon to a depth of 8 cm [8].

The animals were removed from the experiment according to the recommendations under the influence of anesthesia with the drug "Zoletil 100" (INN: tiletamine hydrochloride) (Virbac Sante Animale; France) at a dose of 20 mg/kg.

EC verification was carried out by assessing the clinical picture and morphology of the lesion site in the colon. Suppositories with vitamin D_3 were prepared on the basis of a 10% aqueous solution of vitamin D_3, a mixture of polyethylene glycols with different molecular weights, emulsifier T-2, cremophore RH-40 and coliphore were used as

auxiliary substances. The vitamin D_3 content in each suppository was 1500 IU [9]. Rectal suppositories containing 50 mg of 5-ASA were prepared on the basis of rectal suppositories "Salofalk" (INN: Mesalazine, "Doctor Falk Pharma GmbH", Germany). The size and shape of the suppositories corresponded to the structural features of the distal colon of rats, the final weight of each suppository was 300 mg. The studies were carried out on days 2, 4 and 6.

To assess the clinical status, a Disease activity index (DAI) adapted for rats was used, including such indicators as body weight, stool consistency and blood admixture in feces [10]. The calculation of indicators on a 5-point scale from 0 to 4 was carried out daily, the maximum possible value of DAI was 12. Fragments of the distal part of the colon were fixed in a 10% neutral formalin solution; serial sections were stained with hematoxylin and eosin. The expression of myeloperoxidase (MPO) and TNFα in the colon mucosa was evaluated by immunohistochemical method using sets of rat-specific antibodies ("Cloud. Clon. Corp.", China) and highly adhesive glasses with a positively charged surface (Super Frost Plus); the result was expressed in units/mm^2. The formulation of the reaction was carried out in the immunohistostainer "Bench Mark XT" (Ventana, USA) in compliance with the protocol of the study. The "Ultra VIEW Universal DAB" system (Ventana, USA) and a complex of secondary antibodies and chromogen were used for visualization. In ten randomly selected fields of view on the microscope "Leica DMRXA" (Germany), at an increase of x400, the numbers of neutrophils (NF), lymphocytes (LC), eosinophils (EF), histiocytes (HZ), plasma cells (PC), fibroblasts (FB) were calculated by 1 mm^2, at an increase of x100, the diameter of the ulcer was determined defect (in microns). Morphometry was performed using the ImageScope M program (Russia). Colon tissue damage was assessed on a scale from 0 to 6 with determination of the relative area of damage, intestinal wall thickness, angiogenesis, loss of goblet cells, severity of leukocyte infiltration and calculation of tissue damage index (tissue damage index, TDI).

Statistical processing of the results was carried out using the IBM SPSS Statistics 19 program. The characteristics of the samples are presented in the format "Me ($Q_{0.25}$-$Q_{0.75}$) [Min-Max]", where Me is the median, $Q_{0.25}$ and $Q_{0.75}$ are the values of the lower and upper quartile, respectively, Min is the minimum value of the sample, and Max is the maximum. The significance of the differences between the groups was assessed using the criteria of Kruskal–Wallis, Mann–Whitney.

Results and discussion

With EC on the 2nd day of observation, a body weight deficit is detected, defecation increases, the consistency of feces changes to liquid, the admixture of blood is determined both with a benzidine test and visually. Animals spend less time grooming, and fewer approaches to food and water are recorded. On the 4th and 6th days, the clinical signs become heavier. The DAI index increases significantly on days 2, 4 and 6, its value is 6 days higher ($p > 0.01$), compared with days 4 and 2 (Table 1).

On the 2nd day of EC, during histological examination of the colon wall, ulcers are detected in the lesion, the bottom of which is located in its own plate of the mucosa and in the superficial parts of the submucosal layer, there is also cellular infiltration with edema of the interstitial tissue, venous and capillary fullness, the crypt epithelium in a state of protein dystrophy (Figure 1A, see 3rd page of cover). On the 4th day of EC, ulcerative defects, swelling of the interstitial tissue, fullness with leukostasis and leukodiapedesis, plasma impregnation and swelling of the vascular walls, stroma infiltration persisted (Figure 2A, see 3rd page of cover). In the depth of ulcerative defects, the proliferation of preserved cambial cells of the intestinal glands. On the 6th day of EC, ulcerative defects with cellular detritus, edema and loosening of the interstitial tissue, vascular

TABLE 1. CLINICAL PICTURE IN EC, Me ($Q_{0.25}$-$Q_{0.75}$) [MIN-MAX]

Indicator	Group 1 Intact (n = 7)	Group 2 EC			Group 3 EC + VD_3			Group 4 EC + 5-ASA		
		2nd day (n = 7)	4th day (n = 7)	6th day (n = 7)	2nd day (n = 7)	4th day (n = 7)	6th day (n = 7)	2nd day (n = 7)	4th day (n = 7)	6th day (n = 7)
DAI, c. u.	0	8.00 (3.00-8.00) [3.00-10.00] *	9.00 (7.00-11.00) [6.00-11.00] *	12.00 (12.00-12.00) [8.00-12.00] *	6.00 (6.00-6.00) [5.00-8.00] *	5.00 (5.00-6.00) [5.00-6.00] * #	5.00 (5.00-5.00) [3.00-5.00] * #	6.00 (5.00-6.00) [5.00-6.00] *	5.00 (5.00-6.00) [5.00-8.00] * #	5.00 (3.00-5.00) [3.00-5.00] * # & &&

Note. *, statistically significant ($p < 0.05$) differences with group 1; #, with group 2, $, with group 3; &, with group 4 on day 2; &&, with group 4 for 4 days. EC, experimental colitis; VD_3, vitamin D_3; 5-ASA, 5-aminosalicylic acid.

fullness are visible (Figure 3A, see 3rd page of cover). Between the infiltration sites, proliferation of young fusiform fibroblasts and the initial phenomena of neoangiogenesis are visible, with pronounced epithelization along the edges of ulcerative defects.

Morphometric assessment of the cellular composition of the infiltrate in the focus of colon injury allowed us to establish that on the 2nd, 4th and 6th days of EC, the content of neutrophils (NF), lymphocytes (LC), eosinophils (EO), plasmocytes (PC), histiocytes (HC) and fibroblasts (FB), the area of the ulcerative defect and TDI significantly increases (Table 2). In the dynamics of EC, the number of NF is less for 4 days ($p < 0.01$) than for 2 days, and for 6 days less ($p < 0.01$) than for 2 days; the number of LC is greater for 6 days ($p < 0.01$) than for 2 and 4 days; the number of EF, HZ and PC on the 4th and 6th days is more ($p < 0.01$) than on the 2nd day; the amount of FB on the 4th day is more ($p < 0.01$) than on the 2nd day, on the 6th day more ($p < 0.01$) than on the 2nd and 4th days. The area of the ulcerative defect is larger on days 4 and 6 ($p < 0.01$) than on day 2. As can be seen, the maximum severity of quantitative representation in the NF focus was recorded on day 2, EF, HZ, PC and FB – on day 2 and 4, LC – on day 6 EC.

The content of MPO and TNFα in the colon tissue increases on the 2nd, 4th and 6th days of the experiment. In the dynamics of EC, the MPO content is 6 days lower than on days 2 and 4; the TNFα content is 6 days lower than on days 2 and 4 of the experimental study. The maximum content of MPO and TNFα in the colon in EC is noted on the 2nd day of the experiment.

Under conditions of local application of vitamin D_3 in EC, a change in the clinical picture in animals is observed. Animals become more active, approaches to food and water increase, animals devote time to grooming. Body weight does not decrease, fecal masses are denser, blood in fecal masses is determined only in a benzidine sample. DAI significantly decreases on days 4 and 6 (Table 1). In the dynamics of the study, the values of DAI for the entire day have no differences. DAI on days 2, 4 and 6 significantly differs from DAI in the group of intact animals, which indicates only a partial recovery of the indicator.

In EC, under the conditions of vitamin D_3 use, during histological examination of the colon wall in the lesion on day 2, ulcerative defects were recorded in the own plate of the mucous membrane and the submucosal layer with venous and capillary fullness, the mucous membrane is moderately edematous, the crypts are shortened, expanded, their epithelium is in a state of granular dystrophy (Figure 1B, see 3rd page of cover). On day 4, completely epithelized areas of replacement of ulcerative defects of the mucous membrane with the initial formation of intestinal glands and crypts, focal infiltration by granulocytes, proliferation of young fibroblasts were revealed (Figure 2B, see 3rd page of cover). On day 6, complete epithelialization of ulcerative defects, focal infiltration and extensive fields of proliferating fibroblasts, newly formed connective tissue fibers and vessels in large quantities are observed (Figure 3B, see 3rd page of cover).

Morphometric assessment of the cellular composition of the infiltrate in the focus of colon damage in EC with the use of vitamin D_3 allowed us to establish that on day 2 the number of NP, LC, EF and PC significantly decreases, and the number of HZ and FB increases. On day 4, the number of NF, LC, EF and PC significantly decreases, and the number of FB increases. On day 6, the number of NF, LC, EF and PC significantly decreases, and the number of HZ and FB increases. On days 2, 4 and 6, the area of ulcerative lesions decreases; on days 4 and 6, TDI decreases (Table 2). All morphometric parameters during the whole day of the experiment did not reach the values of the group of intact animals; they were partially restored. In the dynamics of EC, the number of EF and HZ is greater for 4 days ($p < 0.01$) than for 2 days, for 6 days more ($p < 0.01$) than for 2 days, the number of FB is greater for 4 days ($p < 0.01$) than for 2 days, for 6 days more ($p < 0.01$), than on the 4th and 2nd days. TDI on the 4th and 6th days is less ($p < 0.01$) than on the 2nd day. All morphometric parameters during the whole day of the experiment did not reach the values of the group of intact animals, they were partially restored.

Against the background of the use of vitamin D_3 in EC, on the 4th and 6th days of the experiment, the concentrations of TNFα and MPO in the homogenate of the zone of alteration of the colon mucosa decrease, not reaching values in the group of intact animals (Table 3). In dynamics, MPO expression is 4 days less ($p < 0.01$) than on day 2, 6 days less ($p < 0.01$) than on day 4 and 2; TNFα expression is 6 days less ($p < 0.01$) than on day 4 and 2.

Against the background of the use of 5-ASA in EC, its well-known anti-inflammatory properties were recorded. Animals willingly engage in mutual grooming, approaches to food and water are becoming more frequent. On days 2, 4 and 6, the DAI index decreases: body weight stabilizes, animals become more active, diarrhea is replaced by feces, and blood in feces is determined only in a benzidine sample. In the dynamics of EC, the DAI parameter is 6 days lower ($p < 0.01$) than on the 2nd and 4th days of the experiment.

The DAI parameter in the group of animals under the conditions of use of 5-ASA and in the group of animals under the conditions of use of vitamin D_3

TABLE 2. MORPHOMETRIC INDICATORS IN THE FOCUS OF DAMAGE TO THE LARGE INTESTINE IN EC, Me ($Q_{0.25}$-$Q_{0.75}$) [MIN-MAX]

Indicator	Group 1 Intact (n = 7)	Group 2 EC			Group 3 EC + VD_3			Group 4 EC + 5-ASA		
		2nd day (n = 7)	4th day (n = 7)	6th day (n = 7)	2nd day (n = 7)	4th day (n = 7)	6th day (n = 7)	2nd day (n = 7)	4th day (n = 7)	6th day (n = 7)
Neutrophils, un/mm²	204.56 (189.71-223.57) [147.45-231.92]	2651.41 (2558.85-2813.85) [1827.41-3529.41] *	1518.48 (1121.49-2100.00) [1100.11-2727.28] *	1333.33 (1213.34-1608.04) [1008.06-3366.33] *	873.78 (666.67-925.92) [654.21-931.67] * #	550.45 (370.37-1006.03) [198.02-1078.43] * #	654.21 (582.53-804.83) [458.71-873.78] * #	926.81 (571.42-1063.83) [502.51-2824.85] * #	803.57 (737.71-1592.92) [634.92-1872.14] * #	833.07 (706.520-983.607) [645.160-1875.00] * #
Lympho-cytes, un/mm²	338.99 (305.14-368.35) [284.55-368.95]	1104.48 (947.67-1333.34) [931.67-1396.04] *	1004.55 (880.09-1238.11) [841.12-1408.45] *	1667.02 (1302.62-2038.84) [1209.67-2685.18] *	680.07 (511.78-849.32) [427.81-864.63] * #	710.67 (495.06-733.95) [462.96-784.31] * #	642.19 (582.61-891.11) [467.29-904.52] * #	1229.83 (983.61-1259.84) [489.13-2480.00] * $	796.46 (555.56-1095.89) [531.91-1967.21] *	596.51 (452.26-1034.48) [400.00-1129.94] * # &
Eosinophils, un/mm²	146.91 (120.83-176.18) [105.49-187.66]	852.34 (839.46-857.45) [757.57-1456.31] *	2671.29 (2352.95-3553.31) [1313.13-4476.19] *	2380.11 (2110.11-2613.05) [1415.57-3333.33] *	467.29 (304.57-611.13) [297.03-716.47] * #	852.89 (635.86-1094.25) [198.12-1308.41] * # €	805.12 (685.42-867.49) [594.05-901.81] * # €	807.65 (514.28-1105.52) [451.97-1483.51] *	1587.31 (707.960-2232.143) [655.73-2792.79] * #	726.42 (483.87-1475.41) [217.39-1574.81] * #
Histiocytes, un/mm²	13.47 (13.42-13.65) [12.72-13.86]	571.94 (198.02-750.26) [93.45-757.57] *	1197.11 (1049.31-1614.91) [818.83-1714.28] *	1006.03 (970.87-1009.17) [807.26-1262.13] *	913.31 (759.37-1102.82) [710.66-1187.12] * #	1395.36 (1313.13-1600.00) [1207.24-1682.24] * €	1617.79 (1512.09-1809.04) [1202.41-1962.61] * # €	698.91 (351.75-862.06) [338.98-904.25] *	821.91 (655.73-1415.92) [476.19-1648.93] *	1097.96 (800.01-1338.58) [543.47-1696.42] * $
Plasmo-cytes, un/mm²	13.42 (12.87-13.56) [12.72-13.64]	673.13 (549.12-704.52) [535.91-748.66] *	804.02 (713.06-910.01) [605.44-1708.54] *	810.13 (804.82-1210.12) [707.07-1800.00] *	480.81 (370.37-560.74) [366.97-582.52] * #	401.06 (372.67-411.77) [186.91-471.68] * #	373.83 (297.03-545.56) [275.22-588.23] * #	495.84 (201.01-549.45) [169.49-571.42] * #	530.97 (446.42-540.54) [409.83-593.61] * # $	517.52 (480.01-655.73) [163.04-714.28] * #
Fibroblasts, un/mm²	22.66 (13.56-26.82) [13.42-27.28]	512.77 (281.37-711.65) [198.02-748.66] *	1146.77 (866.81-1358.22) [857.44-2285.71] *	1685.27 (1523.84-2057.07) [1388.88-2079.21] *	1821.02 (1817.34-1845.66) [1467.89-1962.61] * #	2353.94 (2311.23-2401.00) [2112.67-2952.38] * # €	2467.89 (2413.88-3047.61) [2304.61-3517.58] * # € €€	856.07 (790.96-1318.68) [653.26-2011.49] * # $	1506.84 (1250.01-1858.41) [1031.74-1981.98] * $	2048.21 (1612.91-2240.01) [1086.95-3214.28] * $ &

Таблица 2 (окончание)
Table 2 (continued)

Indicator	Group 1 Intact (n = 7)	Group 2 EC			Group 3 EC + VD_3			Group 4 EC + 5-ASA		
		2nd day (n = 7)	4th day (n = 7)	6th day (n = 7)	2nd day (n = 7)	4th day (n = 7)	6th day (n = 7)	2nd day (n = 7)	4th day (n = 7)	6th day (n = 7)
Diameter of the ulcerative defect, mcm	0	575.00 (305.00-780.60) [290.33-791.65] *	735.00 (635.52-976.50) [600.75-983.42] *	753.00 (372.00-882.50) [359.42-894.39] *	294.00 (197.00-357.00) [187.35-372.35] * #	242.00 (151.00-539.00) [143.65-537.25] * #	238.50 (169.00-299.00) [161.35-301.95] * #	250.82 (211.35-284.64) [200.75-299.42] * #	492.26 (267.16-564.21) [243.75-574.32] * #	213.49 (183.47-241.85) [181.75-245.75] * #
TDI, c. u.	0	3.71 (3.00-4.00) [2.00-5.00] *	3.57 (3.00-4.00) [2.00-6.00] *	3.42 (3.00-4.00) [3.00-5.00] *	3.00 (3.00-4.00) [2.00-5.00] *	2.17 (1.00-3.00) [1.00-4.00] * # €	2.12 (1.00-3.00) [1.00-4.00] * # €	3.61 (3.00-4.00) [2.00-4.00] *	2.00 (1.00-3.00) [1.00-4.00] * #	1.81 (1.00-2.00) [1.00-3.00] * #

Note. *, statistically significant ($p < 0.05$) differences with group 1; #, with group 2; $, with group 3; €, with group 3 on day 2; €€, with group 3 on day 4; &, with group 4 on day 2; &&, with group 4 for 4 days. EC, experimental colitis, VD_3, vitamin D_3; 5-ASA, 5-aminosalicylic acid, TDI, tissue damage index.

TABLE 3. CONTENT OF MPO AND TNFα IN THE FOCUS OF DAMAGE TO THE LARGE INTESTINE IN EC, Me ($Q_{0.25}$-$Q_{0.75}$) [MIN-MAX]

Indicator, un/mm²	Group 1 Intact (n = 7)	Group 2 EC			Group 3 EC + VD_3			Group 4 EC + 5-ASA		
		2nd day (n = 7)	4th day (n = 7)	6th day (n = 7)	2nd day (n = 7)	4th day (n = 7)	6th day (n = 7)	2nd day (n = 7)	4th day (n = 7)	6th day (n = 7)
MPO	16.28 (7.66-26.81) [5.74-28.73]	1241.37 (967.04-1486.59) [729.88-1708.81] *	938.69 (770.15-1109.19) [760.53-1206.89] *	775.86 (766.28-814.17) [722.22-1427.21] *	1053.64 (977.01-1091.95) [957.85-1206.89] *	498.08 (478.92-593.86) [421.45-670.49] * # €	287.35 (268.19-287.35) [210.70-344.82] * # € €€	984.67 (773.94-1112.83) [634.09-1170.11] *	555.55 (478.92-574.71) [440.61-593.89] * # &	249.04 (210.72-249.04) [191.57-325.67] * # $ & &&
TNFα	52.68 (44.06-63.21) [26.81-76.62]	1321.83 (919.54-1475.09) [720.31-1551.72] *	1302.68 (1264.36-1321.83) [1245.21-1360.15] *	752.87 (703.06-814.17) [670.49-950.19] *	766.28 (670.49-957.85) [632.18-1053.64] *	727.96 (727.97-766.28) [632.18-785.44] * #	210.72 (172.41-229.88) [134.09-268.19] * # € €€	766.28 (545.97-967.43) [498.08-996.16] *	402.29 (344.82-440.61) [306.51-478.92] * # $ &	191.57 (172.41-191.57) [172.41-229.88] * # & &&

Note. *, statistically significant ($p < 0.05$) differences with group 1; # – with group 2; $ – with group 3; € – with group 3 on day 2; €€ – with group 3 on day 4; & – with group 4 on day 2; && – with group 4 for 4 days. EC, experimental colitis; VD_3, vitamin D_3; 5-ASA, 5-aminosalicylic acid.

has no statistically significant differences on the 2nd, 4th and 6th days of observation, which indicates the comparability of the effects of vitamin D_3 and 5-ASA with respect to clinical signs of EC (Table 1).

The clinical picture of EC in the conditions of 5-ASK application is reflected in the morphology of the alteration zone. On day 2, ulcerative defects are located in the superficial parts of the submucosal and the proper plate of the mucous membrane of the colon wall, infiltration in the projection of the defect, protein dystrophy of the glands (Figure 1C, see 3rd page of cover). On day 4, defects are more often found in the own plate of the mucosa, newly formed connective tissue fibers, signs of neoangiogenesis and epithelization of surface defects are revealed (Figure 2C, see 3rd page of cover). On day 6, the depth of defects varies from the superficial to the submucosal layers, infiltration with an admixture of histiocytes and plasmocytes was detected in the submucosal layer, fibrillogenesis, neoangiogenesis, epithelialization and proliferation of cambial cells of intestinal glands were recorded (Figure 3C, see 3rd page of cover).

Morphometric analysis of the colon alteration zone in EC under conditions of local application with 5-ASA found that on day 2 the number of neutrophils and plasmocytes decreases, the number of fibroblasts increases; on day 4 the number of neutrophils, eosinophils and plasmocytes decreases; on day 6 – neutrophils, lymphocytes, eosinophils and plasmocytes (Table 2). The diameter of the ulcerative defect decreases by 2, 4 and 6 days; the TDI indicator decreases by 4 and 6 days. When assessing the content of TNFα and MPO in the cell populations of colon tissue under conditions of local application of 5-ASA, it was revealed that these parameters decrease on days 4 and 6 (Table 3). In the dynamics of EC, the content of MPO and TNFα is 6 days lower than on the 2nd and 4th days of the experiment.

So, with EC, in the conditions of using rectal suppositories with vitamin D_3, unlike 5-ASA, the repair of the ulcerative defect is fixed earlier, the cellular infiltration of the inflammatory focus decreases. When comparing the morphometric parameters of the colon alteration zone in EC under the conditions of rectal suppositories with vitamin D_3, in contrast to the use of 5-ASA, a decrease in the number of lymphocytes, an increase in the number of fibroblasts was revealed on day 2, a decrease in the number of plasmocytes and an increase in the number of fibroblasts on day 4, an increase in the number of histiocytes and fibroblasts on day 6. The diameter of the ulcerative defect and the integral parameter of intestinal tissue damage, the TDI index, have no significant differences between the compared groups. When comparing the effectiveness of rectal suppositories with vitamin D_3, in contrast to the use of rectal suppositories with 5-ASA, the MPO content is higher on day 6, the TNFα content is higher on day 4.

Thus, the analysis performed in EC allows us to talk about the positive effect of the use of original rectal suppositories with vitamin D_3 on clinical manifestations according to the DAI indicator (stabilization of body weight, change in stool consistency to a more decorated one, absence of rectal bleeding) and the morphological picture of the lesion area (reduction of infiltration of the colon wall by neutrophils, lymphocytes, eosinophils and plasmocytes, involved in the alteration of the intestinal wall, an increase in the number of histiocytes and fibroblasts – regulators and participants of the repair, respectively, as well as a decrease in the diameter of the ulcerative defect, a decrease in the TDI index).

The clinical and morphological picture of colon lesions in EC corresponds to changes in IBD in humans and allows the oxazolone model of colitis to be used to study the pathogenesis and test the effectiveness of new therapeutic approaches in IBD [14].

We believe that the results obtained are related to several mechanisms of action of vitamin D_3 in EC. Firstly, the immunotropic effect of vitamin D_3 is realized by the action of the active metabolite of vitamin D_3 calcitriol on the proliferation and differentiation of T lymphocytes, a decrease in Th1, Th17 and an increase in – Treg due to a decrease in the synthesis of IL-1, IL-2, IL-6, IL-12, IL-17, IFNγ and TNFα, increased IL-10 synthesis. Vitamin D_3 inhibits the migration of macrophages and their release of IL-1, IL-6, IL-8, IL-12, chemotaxis and accumulation of neutrophils [11]. Vitamin D_3 interferes with the expression of TLR, CD40, CD80, CD83 and CD86 on the surface of dendritic cells, reduces their secretion of IL-2 and IFNγ, increases the synthesis of IL-10 [12]. This limits the activity of the inflammatory process and the alteration of tissues in the colon [2].

Secondly, vitamin D_3 accelerates repair in the focus of colon damage in EC. When interacting with specific nuclear receptors of colon epithelial cells (VDR), vitamin D_3 increases the expression of vinculin, zonulin, occludin, claudin – proteins involved in the formation of epithelial cells [12]. An increase in the number of histiocytes and fibroblasts in the lesion site indicates the activity of repair processes in the intestinal wall. In addition, the restriction of vascular exudative and leukocyte reactions due to anti-inflammatory and antioxidant effects accelerates the repair under conditions of vitamin D_3 use.

Conclusion

Thus, the use of vitamin D_3 in EC in the composition of original rectal suppositories in a total dose of 18,000 IU reduces the severity of clinical signs, the

representation of cells involved in tissue destruction in the colon wall, increases the representation of cells mediating repair, reduces the content of MPO and TNFα in the colon alteration zone.

The effects of using rectal suppositories with vitamin D_3 on clinical signs, the size of the ulcerative defect, the content of MPO and TNFα in the alteration zone are comparable to the effects of using rectal suppositories with 50 mg of 5-ASA; they are more pronounced with respect to the dynamics of the cellular composition of the colon alteration zone.

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