DOI: 10.2478/cipms-2019-0011 Curr. Issues Pharm. Med. Sci., Vol. 32, No. 2, Pages 57-62

sciendo

Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: http://www.curipms.umlub.pl/

Nitroso-oxidative stress after activation of 5-HT$_4$ receptors under conditions of colitis in rats

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ARTICLE INFO

Received 07 April 2017
Accepted 12 May 2018

Keywords:
experimental colitis,
mosapride,
NO-synthase,
lipid peroxidation,
antioxidant enzymes.

ABSTRACT

Serotonin (5-hydroxytryptamine, 5-HT) plays an important role in the regulation of the functioning of the gastrointestinal tract, including that of the colon. The response of smooth muscles, blood vessels and colon mucosa (CM) to 5-HT is realized through the activation of various types of 5-HT receptors, in particular, 5-HT$_4$ receptors, since the latter are identified on colon cells membranes (enterocytes, smooth muscles and endothelium). The aim of our study was to determine the effect of 5-HT$_4$ receptors agonist (mosapride) on nitrogen (II) oxide production and lipid peroxidation in CM and colon muscle tissue (CMT) under the conditions of experimental ulcerative colitis (UC).

ABBREVIATIONS AND SYMBOLS

CM – colon mucosa, CMT – colon muscle tissue, EC cells – enterochromaffin cells, HT – hydroxytryptamine, MDA – malonic dialdehyde, MPO – myeloperoxidase, NO – nitrogen (II) oxide, NOS – nitrogen (II) oxide synthase, cNOS – constitutive nitrogen (II) oxide synthase, iNOS – inducible nitrogen (II) oxide synthase, SOD – superoxide dismutase, UC – ulcerative colitis.

INTRODUCTION

Development of UC is accompanied by oxidative processes due to the increased concentration of free radicals and the inadequate response of the antioxidant system. This results in different pathological changes in colon cells [1].

Inflammation in the CM and CMT brings about increased production of nitrogen (II) oxide (NO), a gaseous mediator, the main function of which is vasodilatation [2]. NO is produced by different isoforms of NO synthases. These are: inducible (activated by cytokines) and constitutive (endothelial and neuronal) [3]. Colon inflammation is accompanied by the activation of inducible NO-synthase (iNOS) that results in increased synthesis of peroxynitrite, a product of NO, and superoxide radical reaction, causing nitration and nitrosylation of cytoplasmic proteins [4]. Thereby, the increase of nitroso-oxidative processes is an important factor in the mechanism of UC development [5].

During inflammation, the activity of iNOS and cNOS changes [4] and this indirectly indicates changes in cytokines concentration. The effect of myeloperoxidase (MPO), reflecting the presence of neutrophils is similar, it produces hypochlorous acid and tyrosil radicals using hydrogen peroxide. Both of these are cytotoxic compounds and may aggravate lipid peroxidation [6].

The effect of 5-HT as a biologically active substance involved in regulation of colon function, is crucial [7]. The mucous membrane of digestive tract contains about 95% of the total 5-HT reserve of in the rat body. Of this, enterochromaffin cells (EC cells) synthetize 90% [8]. The distribution of EC cells in different parts of the digestive tract is equable – their content is approximately 0.25% when compared to other cells of digestive tract mucosa, but are higher (0.5%) in the area between the colon and rectum [9].

The complex interaction of bacteria, diet and intestinal cells affect synthesis, release and degradation of 5-HT, and therefore all the afore-mentioned factors may be responsible for the altered 5-HT function documented in many gastrointestinal diseases [10]. Indeed, abnormal regulation of 5-HT metabolism in the human gut was noted in numerous gastrointestinal disorders, in particular, in inflammatory bowel disease [11] and functional disorders such as irritable bowel syndrome [12]. Furthermore, alteration in 5-HT signaling was shown to be associated with celiac disease [13], colorectal cancer [14,15] and diverticular disease [16].

Release of 5-HT on neurons and smooth muscles of the CM affects secretion, reabsorption, microhemodynamics and motility [17]. The effect of 5-HT depends on the type of receptor that it binds to after being released from EC cells. Herein, the following types of 5-HT receptors have been

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identified in the colon: 5-HT_{1A}, 5-$HT_{1B/D}$, 5-HT_{1P}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_3, 5-HT_4, 5-HT_7. These are localized on enterocyte neurons, smooth and longitudinal muscles of the colon [18]. Some 5-HT receptors have been found on various immune cells such as B- and T-lymphocytes, monocytes, macrophages [19]. Mast cells, macrophages and T-cells also have the ability to synthesize 5-HT from tryptophan [20-22]. Since it is known from previous studies that 5-HT level increases in CM under conditions of inflammation [10], the role of 5-HT receptors requires deeper elucidation.

Various types of 5-HT receptors regulate the motor function of the colon, and depending on the type of receptor and type of impact (stimulation/blockage), 5-HT is involved in the regulation of muscle relaxation (stimulation of 5-HT_2, 5-HT_7) and contraction (blocking 5-HT_3, stimulation of 5-HT_4 and 5-HT_1 receptors) [7]. Several colon motor abnormalities such as lack of contractility and increase of propulsive contractile waves were investigated in UC patients [23]. Reduction of colon motor function may increase inflammation due to colon dilatation and the arrest of fecal mass with colon bacteria, that when contacting damaged CM, enhances inflammation.

The activation of 5-HT_4 receptors leads to release of acetylcholine from the efferent nerves of enteric nervous system, as well as 5-HT from the EC cells, causing colon muscle contractions [7]. The effect of 5-HT on epithelial cells also involves 5-HT_4 receptors, resulting in the CM increasing production of mucus, water and chlorine ions [24]. Finally, 5-HT signaling via 5-HT_4 receptors leads to visceromotor sensitivity decrease [25]. Thus, 5-HT_4 receptors are involved in regulation of intercellular integration of mucous and muscle tissues of the colon [26].

The aim of our study was to determine the effect of the 5-HT_4 receptors agonist (mosapride) on the processes of cytoprotection in the CM and CMT in experimental UC, exploring the L-arginine/NOS/NO pathway, lipid peroxidation processes, antioxidant enzymes and MPO activity.

MATERIALS AND METHODS

The structure of this study and the experimental procedures performed on the animals were approved by the Ethical Committee of Lviv National Medical University (Protocol №3, 16.03.2015). The experiments were carried out in accordance with international guidelines for the use and care of laboratory animals.

Animals. 40 nonlinear white rats of both sexes, weighing 200-220 g were used. Rats were placed into 3 groups: 1 – intact animals, while UC was modeled in rats of groups 2 and 3. Animals of the 3 group were administered the 5-HT_4 receptors agonist (mosapride) intragastrically and then UC was modeled. Drugs. 5-HT_4 receptors agonist mosapride ("Mosyd MT" tablets, Torrent Pharmaceuticals Ltd., India) was used in this research. Water suspension of tablets was prepared and mosapride was administered intragastrically via an orally introduced polyethylene tube at a single dose 10 mg/kg (volume of 1 mL) 1 hour before UC induction.

Procedure. UC was induced in animals of the 2nd and 3rd groups by introduction of 1 mL of 4% solution of acetic acid into the colon, which was pre-washed by saline. Exposure of acid solution was 1 min, then the colon was washed with 0.1 M phosphate buffer (pH = 7.4) [27]. Euthanasia was performed by intraperitoneal administration of urethane solution (Sigma, Switzerland) in a dose 3 g/kg.

After opening of the abdominal cavity, the colon was removed, cut lengthwise and washed with 0.9% sodium chloride, while the remaining moisture was removed with filter paper. Assessment of area and index of damage were performed by the planimetry method [28].

Lipid peroxidation level was determined due to malonic dialdehyde (MDA) concentration by measurement of the colored complex formed by MDA and thiobarbituric acid in acidic medium after boiling [29]. MPO activity was assessed through reaction of *o*-dianisidine oxidation by hydrogen peroxide, which is a substrate of MPO [30] and was expressed in IU/mg of protein. Superoxide dismutase (SOD) activity was measured by way of reaction of nitro blue tetrazolium reduction and formazan production and was expressed in IU·10^2/mg of protein [31]. Catalase activity was determined due to peroxomolibdate (product of molybdate ammonium oxidation reaction) content and was expressed in μmol H_2O_2/min·mg of protein [32]. The activity of iNOS and cNOS was investigated through *L*-citrulline concentration measurement and was expressed in nmol of *L*-citrulline/min·mg of protein [33]. Arginase activity was assessed via urea concentration measurement and was expressed in μmol of carbamide/min·mg of protein [34]. Nitrites and nitrates concentrations were determined by reaction with Griess reagent and expressed in μmol/L [35]. Absorption was measured on biochemical analyzer StatFax 1904 (USA).

Statistical analysis. Statistical analysis was performed using OriginPro 7.0. Data are presented as the mean and standard deviation. The reliability was determined using the Student`s test because distribution, determined using the Shapiro-Wilk test, was normal.

RESULTS

Effect of acetic acid on CM (UC). Induction of UC led to emergence of deep erosions and ulcers in CM (Fig. 1,2) with the inflammation area 12.4±3.32 cm^2. The damage

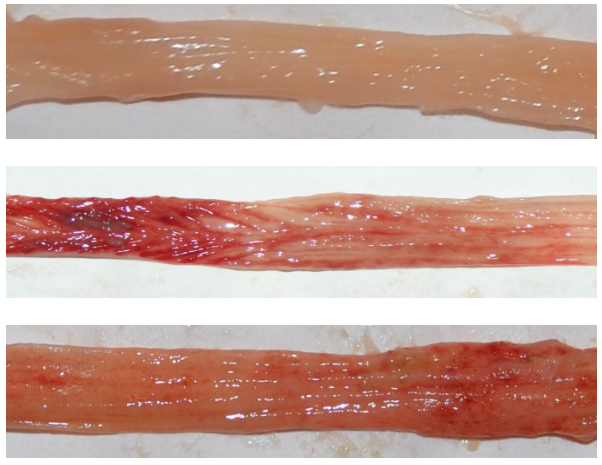

Figure 1. Macroscopic changes of colon mucosa in control group (1) and colitis group (2), after administration of 5-HT_4 receptors agonist – mosapride, under the conditions of colitis (3)

index in the 2 group of animals was 11.3±2.04. Development of UC was accompanied by the increase of MDA concentration by 53% ($p \leq 0.001$), while MPO activity increased 5.6-fold ($p \leq 0.001$) compared to the control group (Tab. 1). In addition, the activity of antioxidant enzymes, SOD and catalase increased. Herein, activity of SOD rose 2.3-fold ($p \leq 0.001$), activity of catalase increased 40% ($p \leq 0.001$) compared to the control group (Tab. 1). The L-arginine/NOS/NO pathway under the conditions of UC was characterized by the following changes: iNOS activity increased 4.3-fold ($p \leq 0.001$) and cNOS activity decreased 29% ($p \leq 0.001$) compared with the control group of animals (Tab. 1). Intensive use of L-arginine by NOS as a substrate led to reduced activity of arginase. This decreased 3.5-fold ($p \leq 0.001$) compared to the control group. Furthermore, the concentration of L-arginine decreased from 94.71±23.71 μmol/L in the control group, to 68.8±11.36 μmol/L in the UC group (27%) compared to control ($p \leq 0.001$). Nitrite and nitrate concentration also increased in the 2nd group of animals. Here, nitrite concentration rose 3.2-fold ($p \leq 0.001$) and the total concentration of nitrite and nitrate increased 1.8-fold ($p \leq 0.001$) compared to the control group (Tab. 1).

Effect of acetic acid on CMT (UC). Similar changes were observed in CMT: concentration of MDA increased 2.1-fold in the 2nd group ($p \leq 0.001$) and MPO activity rose 4.8-fold ($p \leq 0.01$) compared to the control group (Tab. 2). Another ratio of antioxidant enzyme activity was noted: SOD activity increased 60% ($p \leq 0.05$) and catalase activity was enhanced by 87% ($p \leq 0.01$), compared to the control group (Tab. 2). Moreover, activity of iNOS increased 3.4-fold ($p \leq 0.001$) and cNOS activity decreased 17% ($p \geq 0.05$) compared to control (Tab. 2). However, arginase activity did not change significantly (28%) ($p \leq 0.05$) compared to control group (Tab. 2). A different concentration ratio was observed in CMT – nitrite concentration increased 45% ($p \leq 0.05$) and total nitrite and nitrate rose 77% ($p \leq 0.001$) compared to the control group (Tab. 2).

Effect of mosapride on CM (in conditions of UC). Administration of 5-HT_4 receptors agonist (mosapride) under conditions of UC led to decrease of inflammation area to 7.4±1.03 cm^2, or 60% ($p \leq 0.01$) lower compared to the UC group (Fig. 1.3). After administration of 5-HT_4 receptors agonist (mosapride), the damage index decreased to 7.8±2.02 points, that is 69% ($p \leq 0.01$) lower compared to the UC group. In addition, administration of 5-HT4 receptors agonist (mosapride) under conditions of UC resulted in decrease of the concentration of MDA by 22% ($p \leq 0.01$) and MPO activity by 37% ($p \leq 0.05$) compared to the UC group (Tab. 1). Furthermore, activity of antioxidant enzymes decreased simultaneously: SOD by 28% ($p \leq 0.01$) and catalase by 16% ($p \leq 0.01$), compared to the UC group (Tab. 1). The action of the 5-HT_4 agonist under the conditions of UC decreased iNOS activity by 31% ($p \leq 0.05$), while cNOS activity increased by 19% ($p \geq 0.05$) and arginase activity rose 2.8-fold ($p \leq 0.001$) compared to the UC group (Tab. 1). In addition, the concentration of L-arginine increased in the 3rd group by 16%, compared to UC group ($p \geq 0.05$) to 79.9±12.18 μmol/L. Still, nitrite and nitrate concentration did not change significantly – nitrite concentration decreased by 20% and total nitrite and nitrate concentration decreased by 6%, compared with the UC group (Tab. 1).

Effect of mosapride on CMT (in conditions of UC). Changes in CMT were more significant: MDA concentration decreased by 36% ($p \leq 0.01$) and MPO activity decreased by 42% ($p \geq 0.05$) compared to the UC group (Tab. 2). Similar changes were observed in SOD activity: it decreased to 22% ($p \geq 0.05$) and catalase activity diminished by 26% ($p \leq 0.01$) compared to UC group (Tab. 2). Activity of iNOS decreased to 63% ($p \leq 0.001$), cNOS activity increased by 10% ($p \leq 0.001$), whereas arginase activity increased by 14% ($p \geq 0.05$) compared to the UC group (Tab. 2). What is more, nitrite concentration decreased by 10% and total nitrite and nitrate concentration decreased to 36% compared to the UC group (Tab. 2).

Thus, activation of 5-HT_4 receptors led to the reduction of nitroso-oxidative processes and reduced inflammation.

Table 1. Changes of antioxidant enzymes, MPO, NOS, arginase activity, concentration of MDA, L-arginine in CM

	Control group	UC	UC + Mosapride
MDA, μmol/L	195±29.97	298±30.97***	232±23.42##
SOD, IU·102/mg of protein	7,05±1,365	15,91±3,958***	11,39±3,392##
Catalase, μmol H_2O_2/min·mg of protein	18.14±4.173	25.37±2.235***	21.31±3.233##
MPO, IU/mg of protein	4.753±1.721	26.5±2.644***	16.72±5.609#
iNOS, nmol of L-citrulline/min·mg of protein	162±44.15	702±136***	486±75.28#
cNOS, nmol of L-citrulline/min·mg of protein	677±108	482±31.55***	392±139
Arginase, μmol of carbamide/min·mg of protein	0.179±0,0564	0.051±0.0136***	0.141±0.0313###
NO_2^-, μmol/L	0.871±0.1461	2.817±0.7736***	2.263±0.317
NO_2^-+NO_3^-, μmol/L	3.018±0.4989	5.513±0.773***	5.171±1.3387

Remark. p< 0.05 (*), 0.01 (**), 0.001 (***) compared with vehicle group; p< 0.05 (#), 0.01 (##), 0.001 (###) compared with UC

Table 2. Changes of antioxidant enzymes, MPO, NOS activity, concentration of MDA in CMT

	Control group	UC	UC + Mosapride
MDA, μmol/L	133.3±17.44	269±36.1***	208±22.8##
SOD, IU·10^2/mg of protein	21.22±2.92	33.5±7.46*	26.07±9
Catalase, μmol H_2O_2/min·mg of protein	17.2±2.26	32.2±7.42**	23.7±6.05#
MPO, IU/mg of protein	15.5±1.7	74.2±6**	42.8±18.9
iNOS, nmol of *L*-citrulline/min·mg of protein	197.9±32.7	678.3±111.7***	248.5±67.42###
cNOS, nmol of *L*-citrulline/min·mg of protein	475.6±45.7	395±90.7	436.2±79.21###
Arginase, μmol of carbamide/min·mg of protein	0.29±0.04	0.21±0.03*	0.24±0.02
NO_2^-, μmol/L	0.353±0.0376	0.51±0.0254*	0.458±0.0502#
NO_2^-+NO_3^-, μmol/L	2.65±0.66	4.68±1.84***	2.99±0.58#

Remark. p<0.05 (*), 0.01 (**), 0.001 (***) compared with vehicle group; p<0.05 (#), 0.01 (##), 0.001 (###) compared with UC

DISCUSSION

Our research shows that the effect of the 5-HT_4 receptors agonist (mosapride) led to

the reduction of destructive damage area and decrease of nitroso-oxidative stress under conditions of experimental UC. Herein, the activity of iNOS decreased after activation of 5-HT_4 receptors, that, respectively, led to the decrease of peroxynitrite production. These changes are quite obvious, as the increase of iNOS activity is mediated by the high content of inflammatory cytokines in the area of inflammation [36].

UC is characterized by destructive changes in CM, accompanied by increased content of MDA, protein and mRNA expressions of TNF-α [37], NF-κBp65 [38], IL-1β, IL-17 [39], IL-2, IL-4, IL-5 [40], increased activity of SOD and MPO [41], increased concentrations of prostaglandin E_2 and leukotriene B_4, decreased total glutathione content [42], increased iNOS and COX-2 expression [43], as well as decreased activity of arginase due to bacterial lipopolysaccharides and flagellin admission, in addition to increased oxidative processes in the CM [44]. What is more, increased activity of iNOS under conditions of UC causes reduction of L-arginine concentration and arginase activity [45], indicating the redistribution of *L*-arginine utilization by NOS and arginase under conditions of inflammation.

Unidirectional changes of nitroso-oxidative processes were also observed both in CM and CMT under conditions of UC. Here, increased lipid peroxidation processes and toxic products of NO cause the development of destructive lesions in the CM and CMT dysmotility.

Acute increase of iNOS activity in smooth muscle locally in the areas of inflammation brings about dilatation and reduced motility [46]. This is facilitated by the presence of destructive lesions in the CM, accompanied by the growth of toxic substance permeability from the gut cavity into tissues and blood flow.

Another marker of inflammation is MPO activity, in our experiment, this increased 5.6-fold in CM and 4.8-fold in CMT under conditions of UC, compared to the control group. This was due to the activation of tissue neutrophils.

The substance iNOS, has a significant role in the development of UC. In our work, its activity grew 4.3-fold times in the CM and 3.4-fold in CMT and the effect was accompanied by nitrite anion production and the total number of nitrites and nitrates was affected.

Development of UC is accompanied by increased levels of 5-HT biosynthesis in EC cells in the CM [10]. Here, 5-HT released from EC cells affects motility and secretion, shows neuroprotective effect and regulates cell growth and the protective mechanisms that are involved in the inflammatory process through a paracrine mechanism of action [47]. Of note, in literature, an increased number of EC cells in the CM under conditions of UC was reported [48].

Despite the increase in the allocation of 5-HT from EC cells under UC conditions, colon motor function decreases in the area of inflammation due to smooth muscle dilatation induced by overproduction of NO [36].

The 5-HT4 receptors play a leading role in the regulation of motility, secretion and nociception of colon. They are located on smooth muscles, EC cells and on neurons of the enteric nervous system. Activation of the myenteral neuron 5-HT_4 receptors brings about an increase of acetylcholine release and the activation of the motility, while the effect of 5-HT_4 agonists on EC cell receptors induces the excretion of 5-HT in the extracellular space [49].

The anti-inflammatory, antioxidant and cytoprotective action of 5-HT_4 receptors agonist mosapride involves several mechanisms. Among these are the direct influence of mosapride on cells, the reduction of cytokines production in the CM and the release of local biologically active substances (prostaglandin E_2, histamine, vasoactive intestinal polypeptide) [50]. Increased proliferative processes due to increased content of growth factors can be one of the possible mechanisms of the cytoprotective effect of 5-HT_4 receptors agonist. Increased motility may also be due to reduced bacterial lipopolysaccharide and flagellin admission. The factors that increase cytoprotection may also include enhanced mucus production by epithelial cells on the membrane on which 5-HT_4 receptors are located [7]. Hence, the activation of 5-HT_4 receptors under conditions of acetic acid induced UC, showed cytoprotective, antioxidant and anti-inflammatory effects and reduced nitroso-oxidative processes due to the involvement of different mechanisms of action.

CONCLUSIONS

1. UC manifested with destructive changes in CM, accompanied by the acute increase of MDA concentration and, consequently, lipid peroxidation processes, MPO, iNOS activity, while the activity of arginase decrease in CM and CMT indicates the dominant role of nitroso-oxidative processes in the development of structural damage in the CM in UC.
2. Intragastric administration of 5-HT_4 receptors agonist (mosapride citrate, 10 mg/kg) reduced the severity of destructive lesions of CM, and minimized MDA concentration, MPO, iNOS activity, as well as the activity of antioxidant enzymes – SOD and catalase, indicating the reduction of nitroso-oxidative processes in colon, compared to the UC group.
3. Application of 5-HT_4 receptors agonist mosapride citrate had cytoprotective, antioxidant and anti-inflammatory effects and can diminish nitroso-oxidative stress under conditions of acetic acid induced colitis in rat CM and CMT.

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REFERENCES

1. Takagi T, Naito Y, Yoshikawa T. Free radicals in inflammatory bowel disease. Free Radical Biology in Digestive Diseases. *Front Gastrointest Res. Basel, Karger.* 2011;29:128-36.
2. Kolios G, Valatas V, Ward SG. Nitric oxide in inflammatory bowel disease: a universal messenger in an unsolved puzzle. *Immunology.* 2004;113(4):427-37.
3. Forstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J.* 2012;33(7):829-37.
4. Kruidenier L, Kuiper I, Lamers CB, Verspaget HW. Intestinal oxidative damage in inflammatory bowel disease: semiquantification, localization, and association with mucosal antioxidants. *J Pathol.* 2003;201(1):28-36.

5. Garud S., Peppercorn MA. Ulcerative colitis: current treatment strategy and future prospects. *Therap Adv Gastroenterol.* 2009;2(2): 99-108.
6. Savenkova ML, Mueller DM, Heinecke JW. Tyrosyl radical generated by myeloperoxidase is a physiological catalyst for the initiation of lipid peroxidation in low density lipoprotein. *J Biol Chem*. 1994; 269(32):20394-400.
7. De Ponti F. Pharmacology of serotonin: what a clinician should know. *Gut*. 2004;53(10):1520-35.
8. El-Merahbi R, Loffler M, Mayer A, Sumara G. The roles of peripheral serotonin in metabolic homeostasis. *FEBS Lett*. 2015;589(15):1728-34.
9. Schimmack S, Svejda B, Lawrence B, Kidd M, Modlin I. The diversity and commonalities of gastroenteropancreatic neuroendocrine tumors. *Langenbeck's Arch Surg*. 2011;396:273-98.
10. Manocha M, Khan WI. Serotonin and GI disorders: an update on clinical and experimental studies. *Clin Transl Gastroenterol*. 2012;3(4):e13.
11. Magro F, Vieira-Coelho MA, Fraga S, Serrao MP, Veloso FT, Ribeiro T et al. Impaired synthesis or cellular storage of norepinephrine, dopamine, and 5-hydroxytryptamine in human inflammatory bowel disease. *Dig Dis Sci*. 2002;47(1):216-24.
12. Faure C, Patey N, Gauthier C, Brooks EM, Mawe GM. Serotonin signaling is altered in irritable bowel syndrome with diarrhea but not in functional dyspepsia in pediatric age patients. *Gastroenterol*. 2010;139(1):249-58.
13. Coleman NS, Foley S, Dunlop SP, Wheatcroft J, Blackshaw E, Perkins AC et al. Abnormalities of serotonin metabolism and their relation to symptoms in untreated celiac disease. *Clin Gastroenterol Hepatol.* 2006;4(7):874-81.
14. Ataee R, Ajdary S, Zarrindast M, Rezayat M, Hayatbakhsh MR. Anti-mitogenic and apoptotic effects of 5-HT1B receptor antagonist on HT29 colorectal cancer cell line. *J Cancer Res Clin Oncol.* 2010; 136(10):1461-9.
15. Coogan PF, Strom BL, Rosenberg L. Antidepressant use and colorectal cancer risk. *Pharmacoepidemiol Drug Saf.* 2009;18(11): 1111-4.
16. Costedio MM, Coates MD, Danielson AB, Buttolph TR 3rd, Blaszyk HJ, Mawe GM, et al. Serotonin signaling in diverticular disease. *J Gastrointest Surg.* 2008;12(8):1439-45.
17. Terry N, Margolis GK. Serotonergic mechanisms regulating the GI tract: experimental evidence and therapeutic relevance. *Handb Exp Pharmacol*. 2017;239:319-42.
18. Mawe1 GM, Hoffman JM. Serotonin signaling in the gastrointestinal tract: functions, dysfunctions, and therapeutic targets. *Nat Rev Gastroenterol Hepatol*. 2013;10(8):473-86.
19. Cloez-Tayarani I, Changeux JP. Nicotine and serotonin in immune regulation and inflammatory processes: a perspective. *J Leukoc Biol.* 2007;81(3):599-606.
20. Kushnir-Sukhov NM, Brown JM, Wu Y, Kirshenbaum A, Metcalfe DD. Human mast cells are capable of serotonin synthesis and release. *J Allergy Clin Immunol.* 2007;119(2):498-9.
21. Nakamura K, Sato T, Ohashi A, Tsurui H, Hasegawa H. Role of a serotonin precursor in development of gut microvilli. *Am J Pathol.* 2008;172(2):333-4.
22. O'Connell PJ, Wang X, Leon-Ponte M, Griffiths C, Pingle SC, Ahern GP. A novel form of immune signaling revealed by transmission of the inflammatory mediator serotonin between dendritic cells and T cells. *Blood*. 2016;107(3):1010-7.
23. Bassotti G, Antonelli E, Villanacci V, Baldoni M, Dore MP. Colonic motility in ulcerative colitis. *United European Gastroenterol J.* 2014; 2(6):457-62.
24. Spohn SN, Bianco F, Scott RB, Keenan CM, Linton AA, O'Neill CH et al. Protective actions of epithelial 5-hydroxytryptamine 4 receptors in normal and inflamed colon. *Gastroenterology.* 2016;151(5):933-44.
25. Hoffman JM, Tyler K, Maceachern SJ, Balemba OB, Johnson AC, Brooks EM et al. Activation of colonic mucosal 5-HT4 receptors accelerates propulsive motility and inhibits visceral hypersensitivity. *Gastroenterology.* 2012;142(4):844-54.
26. Kim HS. 5-hydroxytryptamine 4 receptor agonists and colonic motility. *J Smooth Muscle Res*. 2009;45(1):25-9.
27. Fedorak RN, Empey LR, MacArthur C, Jewell LD. Misoprostol provides a colonic mucosal protective effect during acetic acid-induced colitis in rats. *Gastroenterology.* 1990;98(3):615-25.
28. Fomenko IS, Bondarchuk TI, Biletska LP, Panasyuk NB, Sklyarov OY. Study of role of NO-synthase system in gastric mucosa of rats under the influence of non-steroidal anti-inflammatory drugs on the background of adrenaline-induced stress. *Bulletin of Problems in Biology and Medicine*. 2013;1(3):245-9. (in Ukrainian)
29. Timirbulatov MA, Seleznyov EI. Increase intensity method of free oxidation lipid containing blood components and it`s diagnostic value. *Laboratory Work*. 1981;4:209-11. (in Russian)
30. Bradley PP, Christensen RD, Rothstein G. Cellular and extracellular myeloperoxidase in pyogenic inflammation. *Blood*. 1982;60:618-22.
31. Goryachkovskyy AM. *Clinical Biochemistry.* Second edition, corrected and updated. Odessa; 1998:368. (in Russian)
32. Korolyuk MA, Ivanova LI, Mayorova IG. The method of catalase activity determination. *Laboratory Work*. 1988;1:16-9. (in Russian)
33. Ravaeva MY, Chuyan EN. Changes in activity of nitric oxide synthesis system after the action low mm radiation. *Scientific Notes of Tavria National University named after V. I. Vernadsky, Series "Biology, Chemistry"*. 2011;24(4):201-10. (in Russian)
34. Davis RH., Mora JJ. Utilization of exogenous and endogenous ornithine by Neurospora crassa. *Bacteriology.* 1968;96:383-8.
35. Kiselyk IO, Lutsyk MD, Shevchenko LY. Features of the definition of nitrates and nitrites in the peripheral blood of patients with viral hepatitis and jaundice syndrome other etiologies. *Laboratory diagnostics*. 2001;3:43-5. (in Ukrainian)
36. Mourelle M, Vilaseca J, Guarner F, Salas A, Malagelada JR. Toxic dilatation of colon in a rat model of colitis is linked to an inducible form of nitric oxide synthase. *Am J Physiol.* 1996;270:G425-30.
37. Vezza T, Algieri F, Rodriguez-Nogales A, Garrido-Mesa J, Utrilla PM, Talhaoui N et al. Immunomodulatory properties of Olea europaea leaf extract in intestinal inflammation. *Mol Nutr Food Res*. 2017;61(10):1601066.
38. Kang JH, Choi S, Jang JE, Ramalingam P, Ko YT, Kim SY et al. Wasabia japonica is a potential functional food to prevent colitis via inhibiting the NF-κB signaling pathway. *Food Funct.* 2017;8(8):2865-74.
39. Islam J, Koseki T, Watanabe K, Ardiansyah, Budijanto S, Oikawa A et al. Dietary supplementation of fermented rice bran effectively alleviates dextran sodium sulfate-induced colitis in mice. *Nutrients*. 2017;9(7):E747.
40. Shin SK, Cho JH, Kim EJ, Kim EK, Park DK, Kwon KA et al. Anti-inflammatory and anti-apoptotic effects of rosuvastatin by regulation of oxidative stress in a dextran sulfate sodium-induced colitis model. *World J Gastroenterol*. 2017;23(25):4559-68.
41. Zhao L, Xiao HT, Mu HX, Huang T, Lin ZS, Zhong LLD et al. Magnolol, a natural polyphenol, attenuates dextran sulfate sodium-induced colitis in mice. *Molecules*. 2017;22(7):E1218.
42. Nieto N. Experimental ulcerative colitis impairs antioxidant defense system in rat intestine. *Dig Dis Sci*. 2000;45(9):1820-7.
43. Dong WG, Mei Q, Yu JP, Xu JM, Xiang L, Xu Y. Effects of melatonin on the expression of iNOS and COX-2 in rat models of colitis. *World J Gastroenterol.* 2003;9(6):1307-11.
44. Sklyarov AY, Panasyuk NB, Fomenko IS. Role of nitric oxide synthase and cyclooxygenase/lipooxygenase systems in development of experimental ulcerative colitis. *J Physiol Pharmacol.* 2011;62(1):65-73.
45. Cross RK, Keith T, Wilson MD. Nitric oxide in inflammatory bowel disease. *Inflamm Bowel Dis*. 2003;9(3):179-89.
46. Kochar NI, Chandewal AV, Bakal RL, Kochar PN. Nitric oxide and the gastrointestinal tract. *Int J Pharm*. 2011;7(1):31-9.
47. Margolis KG, Stevanovic K, Li Z, Yang QM, Oravecz T, Zambrowicz B et al. Pharmacological reduction of mucosal but not neuronal serotonin opposes inflammation in mouse intestine. *Gut*. 2014;63(6): 928-37.
48. O'Hara JR, Ho W, Linden DR, Mawe GM, Sharkey KA. Enteroendocrine cells and 5-HT availability are altered in mucosa of guinea pigs with TNBS ileitis. *Am J Physiol Gastrointest Liver Physiol.* 2004;287(5):G998-1007.

49. Mawe GM, Hoffman M. Serotonin signaling in the gastrointestinal tract: functions, dysfunctions and therapeutic targets. *Nat Rev Gastroenterol Hepatol*. 2013;10(8):473-86.

50. Sklyarov OY, Kosyy YR, Sklyarov YY. *Fundamentals of Gastroenterology*. Lviv: Quartus; 2011:28. (In Ukrainian)