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Pyrrole Derivatives' Effect on Rats' Colon Mucosa in Experimental Colitis.

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ABSTRACT

We've studied individual and joint effect of pyrrole derived cytotoxic drugs i.e.1-(4-Cl-benzyl)-3-Cl-4-(CF_3-phenylamino)-1H-pyrrol-2,5-dione (MI-1) and 5-amino-4-(1,3-benzothyazol-2-yl)-1-(3-methoxyphenyl)-1,2-dihydro-3H-pyrrol-3-one (D1) on the morpho-functional state of colon mucosa in rats with experimental colitis. It was found that M1 was more effective and less toxic to mucosa as compared to D1 and a combination of D1and MI-1.

Keywords: mucosa, bowel, pyrrole derivatives, experimental colitis.

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INTRODUCTION

Inflammatory bowel diseases (IBD), ulcerative colitis (UC) belongs to, are one of the most serious and unresolved problems in contemporary gastroenterology. The severity and incidence of IBD complications take one of the leading places in GIT structure of diseases [1, 2, 3]. UC is an inflammatory recurrent chronic colonopathy that is known for definitive dystrophic and atrophic mucosa changes accompanied by secretory, motor and digestive disorders [4]. The ethology of this disease is still unknown, thus the reasons thereof are usually of hereditary nature, allergic reactions, nourishing, long-term intake of nonsteroidal anti-inflammatory drugs, etc. [2, 4]. The consequence of chronic UC is a risk of development of malignant formations i.e. colorectal carcinoma, which was confirmed by epidemiological studies [1, 5-7], and thus one may consider UC to be a precancerous state.

It is known that tumor growth is often accompanied by the signs of inflammation in both tumor sites and in adjacent roughly normal tissue [8-10]. The study of long-term intake of nonsteroidal anti-inflammatory drugs in IBD showed a significant decrease in the incidence of colorectal cancer in the target group [5,11,12]. It was also established that signaling inflammatory and carcinogenesis sites shared common links, NFkB in particular [13]. Thus, the blockade of signaling sites that contribute to the development of inflammation may influence the initiation and/or progression of tumors, including colorectal, and conversely, inhibition of signaling sites that are activated in carcinogenesis, cannot but effect the development of inflammation [13,15].

Our previous studies [8-10,16-20] established that pyrrole derived cytotoxic drugs i.e.1- (4-Cl-benzyl)-3-Cl-4-(CF_3-phenylamino)-1H-pyrrol-2,5-dione (MI-1) and 5-amino-4-(1,3-benzothyazol-2-yl)-1-(3-methoxyphenyl)-1,2-dihydro-3H-pyrrol-3-one (D1) *in silico* synthesized at Research and Production Biochemical Center of Taras Shevchenko National University as targeted inhibitors of protein kinase [21] showed antitumor action in chemically induced colon cancer in rats [22]. It was also defined that these compounds contributed to reduction of inflammatory process that led to carcinoma and oxidative stress development [16, 23-25]. Thus, the study of pyrrole derivatives' effect on the development of experimental colitis is perspective both from the point of view of expanding the ideas on the mechanisms of action of aforementioned componds and their application in precancer states to prevent malignification of colon mucosa cells.

The **goal of the** research was to study the effect of pyrrole derivatives on colon mucosa in rats with experimental colitis.

MATERIALS AND METHODS

The study was carried out on non-linear white male rats, initially weighing 207 g, which were kept in standard vivarium conditions. All the work was conducted in accordance with the principles of bioethics, the legislation and regulations of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), General ethics of animal experimentation adopted by the First National Congress of Bioethics (Kyiv, 2001).

The experiment lasted 2 weeks. The experimental model of ulcerative colitis was reproduced by double rectal administration of 1 ml of 4% acetic acid solution with 1 week interval. 10-15 minutes before administration of acetic acid they've purified animals' colon via rectal administration of 2-3 ml of saline solution, which was followed by lower abdomen massage to facilitate emptying. 2.7 mg/kg of MI-1 and 2.3 mg/kg of D1 dissolved in sunflower oil containing 15% of DMSO (total 0.1 ml) was *per os* administered on daily basis. The first administration of compounds was performed 2 hours after the first administration of acetic acid. Control animals were given appropriate solvents. 5 groups (n = 7) were formed, namely: I - control, II - experimental colitis, III - colitis + D1, IV - colitis + MI-1, V - colitis + D1 + MI-1. Rats were sacrificed 1 day after the last administration of substances by CO_2 inhalation and subsequent cervical dislocation.

For histological tests, we took colon (descending colon) segments that were fixed in 10% neutral formalin salt, made paraffin sections and dyed with hematoxylin-eosin by standard method [26]. The specimens were light-optically analyzed with Olympus BX41 (Olympus Europe GmbH, Japan) microscope; color micrographs were taken by Olympus C-5050 Zoom (Olympus Europe GmbH, Japan) digital camera and aforestated microscope. They assessed the overall condition of descending colon mucosa, calculated relative

number of goblet cells and mitotic index of cells in crypts. Morphometric studies were performed using WCIF ImageJ program. Also, they measured mucosa thickness, the depth and width of crypt, the height of colonocytes and cross-sectional area of goblet cells and colonocytes' nuclei.

Statistical analysis of the experimental results were carried out by variation statistics methods [27] using SPSS 16.0 software package, namely: the data were tested for normality of distribution using Z-Kolmogorov-Smirnov test, intergroup comparison was performed using univariate analysis of variance (ANOVA) with posteriori multiple comparisons for F-Fisher criterion, as well as via U-Mann-Whitney test. The difference between values under comparison was deemed to be significant at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Descending colon mucosa in control group rats was of typical histological structure, which was specific for this type of experimental animals, with no signs of pathological conditions. See morphometric data in **Fig. 1-4.**

The impact of induced colitis on rat's descending colon mucosa

Rats with induced colitis had disheveled hair, "wet tail", unformed bowel movements that are obvious signs of diarrhea. In addition, histological studies of colon specimens showed visible diffuse desquamation of surface mucosa epithelium, edema and lymph infiltration of lamina propria. Crypts were filled with mucus, while their inputs were filled with epithelium decay products. There were changes in the vascular bed, which manifested by severe congestion, leukocytes migration, diapetic hemorrhage and blood stasis. Serous layer also showed inflammation, swelling, dilation of blood capillaries and sometimes bleeding. No any significant changes in morphometric parameters as compared to the control group were observed, suggesting that there were no any atrophic changes in mucosa cells. According to the literature, these phenomena suggest the development of colitis without atrophy [1, 3, 7, 28].

D1 impact on descending colon mucosa of rats in ulcerative colitis

The action of D1 on descending colon mucosa resulted in diffuse desquamation and slight dystrophy of surface epithelium. There were significant changes in microvasculature, namely: congestion, blood stasis in capillaries and hemorrhages. Hemorrhages were of different shapes and sizes (ecchymoses) and developed directly from the mucous membrane until submucosa. However, bleeding could have been induced not my D1 action, but by mucosa destruction caused by chemical burns, while inflammation was a reaction to these burns. Also they've observed slight accumulation of polymorphonuclear leukocytes and lymphocytes and histiocytes in submucous layer. There was lymph infiltration in serosa. Morphometry data analysis showed significant increase of mucosa thickness by 31.8% (**fig.1**) as compared to the control group, which might be associated with activation of protective processes in colon mucosa. D1 also contributed to disappearance of colonocytes' atypia signs and mucosa surface thickening, but did not affect the intensity of inflammation and epithelial desquamation.

These data suggest that 2 weeks exposition to D1 in experimental colitis causes mucosa changes manifesting in the form of inflammation, microvasculature disorders (hyperaemia) and lymphatic tissue proliferation (increase in the number of T- and B-lymphocytes), i.e. changes similar to those occurring in experimental colitis [1, 3, 7, 28].

MI-1 impact on descending colon mucosa of rats in ulcerative colitis

The action of MI-1 in experimental colitis resulted in aggregation of lymphocytes in colon mucosa lamina propria, slight disorders in the vascular bed such as erythrostasis and minor diapetic hemorrhage. Also, MI-1 action contributed to the disappearance of surface epithelium desquamation, decreased mucosa inflammation (as compared to experimental colitis group). Morphometric mucosa parameters did not differ significantly from controls. These changes suggest that MI-1, a pyrrole derivative, in experimental colitis inhibits inflammation and contributes to maintaining the integrity of colon mucosa surface epithelium.

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Thus, MI-1, a cytostatic compound, in colitis showed anti-inflammatory and protective properties against colon mucosa, which was consistent with our previous data [16,19,20], and therefore was promising tool for the treatment of inflammatory bowel diseases.

Joint action of MI-1and D1 on descending colon mucosa of rats in ulcerative colitis

Joint action of pyrrole derivatives under study in colitis caused changes in descending colon mucosa of rats similar to those observed in induced colitis without any other interventions, namely: diffuse desquamation of surface epithelium, lamina propria edema, congestion and blood stasis in capillaries. Also, there was a thickening in muscular layer, which could be a sign of intestinal wall spasm. Serous layer showed bleeding, blood clots and lymph infiltration. Morphometry data analysis showed significant increase in mucosa thickness by 34.4% and reduction in mitotic index of crypts' cells by 32.6% (**Fig. 1, 4**), witnessing of the activation of protective processes in the mucous membrane and inhibition of cells proliferation. Thus, joint action of compounds in colitis showed that the severity of mucosa inflammation and epithelial desquamation remained at colitis group level, which meant that no any therapeutic effect was observed. Similar changes were specific of colitis+D1 group, which in its turn meant that D1 effects dominated in joint action of D1 and MI-1.

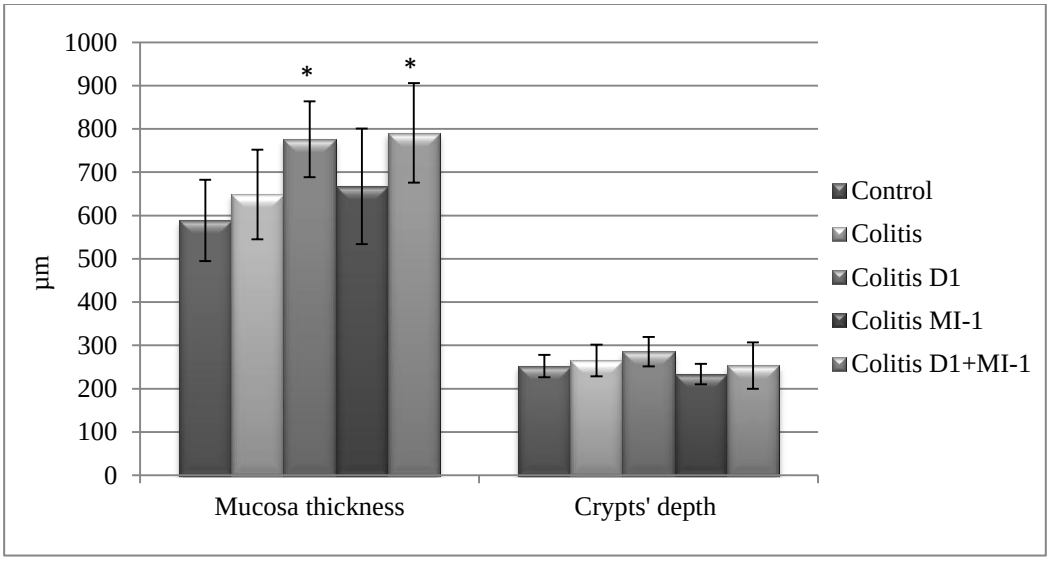

Figure 1: Rats' descending colon mucosa thickness and depth of crypts exposed to D1 and MI-1 in ulcerative colitis.

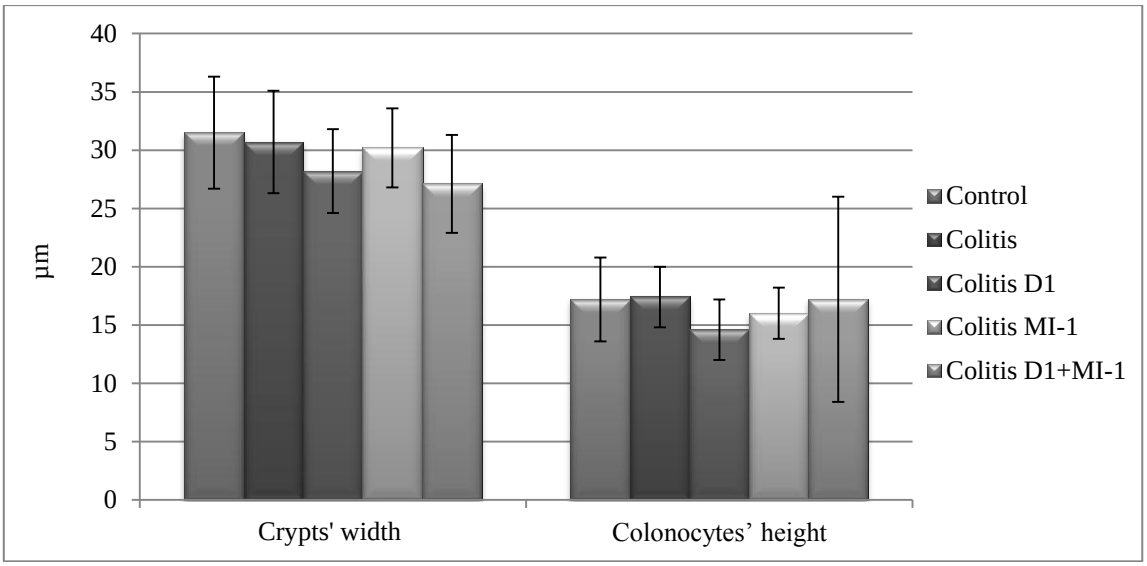

Figure 2: Rats' descending colon crypts' width and colonocytes' height exposed to D1 and MI-1 in ulcerative colitis.

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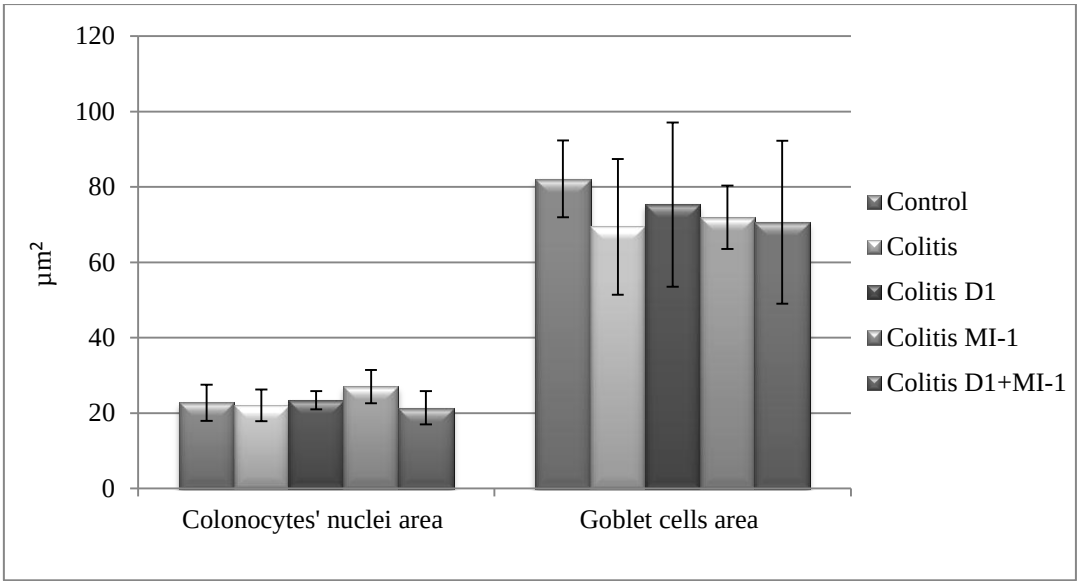

Figure 3: Rats' descending colon colonocytes' nuclei area and goblet cells area exposed to D1 and MI-1 in ulcerative colitis.

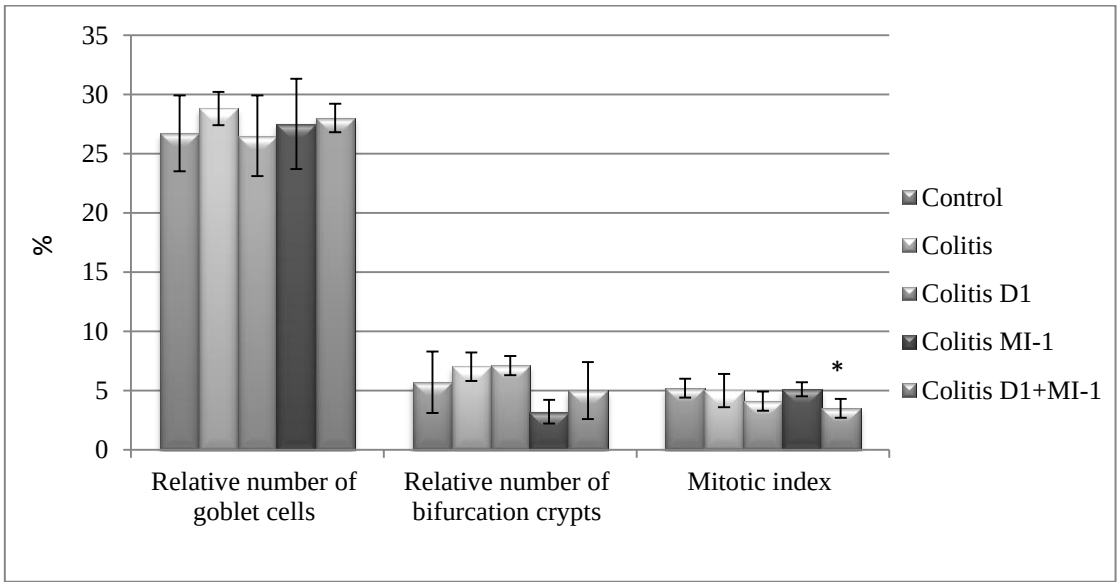

Figure 4: Mitotic index, relative number of goblet cells and bifurcation crypts in ulcerative colitis treated with D1 and MI-1.

CONCLUSIONS

- MI-1 pyrrole derivative in experimental colitis reduced the severity of descending colon mucosa inflammation and contributed to the disappearance of surface epithelium desquamation, which means that it exerted therapeutic action in inflammatory bowel disease.
- D1 pyrrole derivative in colitis did not affect descending colon mucosa surface epithelium desquamation and its inflammation, but contributed to the disappearance of colonocytes' atypia and mucosa surface thickening.
- Joint action of D1 and MI-1 in colitis demonstrated descending colon mucosa changes similar to those observed under individual action of D1; also they've observed inhibition of cells proliferation.
- Maximum anti-inflammatory and protective effects on descending colon mucosa in experimental colitis were observed in MI-1 pyrrole derivative, while protective action of D1 and D1 and MI-1 combination were significantly weaker.

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