ORIGINAL ARTICLE

CONTENTS

Blood serum interleukins levels and their relationship with colon tissue prostanoids in experimental ulcerative colitis

Iryna Vasylieva[1], Oksana Nakonechna[1], Liudmyla Popova[2], Natalia Yarmysh[1], Anastasiia Bezrodna[1], Nataliia Pustova[1]

[1] KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

[2] KYIV MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Aim: To study IL-1β, IL-6, IL-8, IL-10 blood serum levels and their relationship with colon tissue prostanoids in experimental ulcerative colitis.

Materials and Methods: Blood serum interleukins and colon tissue prostanoids were determined by enzyme immunosorbent assay on three groups of WAG population rats (1st control group – rectal saline injection; 2nd control group – 50% ethanol injection; experimental group – injection of 2,4-dinitrobenzene-sulfonic acid in 50% ethanol).

Results: The increased levels of all interleukins both in 2nd control and experimental groups versus 1st control group were found. No differences in IL-6, IL-8 levels between experimental and 2nd control groups were revealed. The relationships between interleukins and PGF2α, between interleukins and TXB2 in all groups, and the appearance of correlations between interleukins and PGE2, between interleukins and PGI2 in experimental group were observed. Negative correlations between IL-8 and COX-2 in 1st control, between IL-8 and COX-1 in both 2nd control and experimental groups were found. Negative correlation between 8-epi-PGF2α and IL-8, positive correlation between 8-epi-PGF2α and PGF2α in experimental group were revealed.

Conclusions: Results obtained testify the possible role of PGF2α and 8-epi-PGF2α in the development of ulcerative colitis and justify the importance of research on the link of 8-epi-PGF2α, IL-8, PGF2α for understanding the mechanisms of development and progression of this disease. Increasing blood serum IL-8 level may be used as marker of reducing colon tissue COX-1 content in both ethanol- and 2,4-dinitrobenzenesulfonic acid-induced ulcerative colites.

KEY WORDS: Interleukins, prostanoids, ulcerative colitis, rats

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INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease are the most common inflammatory bowel diseases (IBD), but their pathogenetic mechanisms are not fully understood.

Immune system mediators play a significant role in IBD development but literature data regarding their changes and effects in disease progressing some times are contradictory. It is believed that proinflammatory cytokines, including IL-1β, IL-6 and others, play significant role in upregulation, while antiinflammatory ones, including IL-10, play significant role in downregulation of disease progression [1]. According Impellizzeri D. et al. (2018) IL-1β knockout mice were hyporesponsive to 2,4-dinitrobenzenesulfonic acid (DNBS)-induced colitis versus wild type mice [2]. However growing evidence has indicated that IL-1β can support colonic homeostasis and attenuate colonic inflammation [3]. According results of Cominelli F. et al. (1990) pretreatment with IL-1β low dose suppressed colonic inflammation in rabbits via PGE2 production [3]. This is consistent with the opinion of Park Y.S. (2007) that endogenous prostanoids are involved in protecting mucous membrane from colon ulcers in dextran sodium sulfate (DSS)-induced inflammation [4]. On the contrary, according to the results of Lejeune M. et al. (2010) apical exposure of T84 colonic epithelial monolayer to PGE2 high levels diminished barrier integrity which was reversed by E-prostanoid 4 receptor antagonist [5].

According some authors IL-10 plays key role in controlling intestinal inflammation. IL-10-deficient mice and patients with mutations in IL-10 or its receptor show increased susceptibility to IBD [6]. In UC patient IL-6 and TNF-α levels were increased, while IL-10 and IL-4 levels were decreased in peripheral blood versus healthy group [7]. On the contrary, data obtained by Melger S. et al. (2003) indicate generalized activation of IL-10 producing CD4+ T cells along the gut of UC patients [8]. In the work of Carrasco A. et al. (2022) it was also revealed an increase in IL-10 messenger RNA (mRNA) in mucosa and IL-10 in blood

in UC patients versus control [9]. Moreover, according to the results of Wang S. et al. (2020) high IL-10 level at the remission phase was associated with shorter duration of remission [10].

Thus, the contradiction of some data regarding interleukines (ILs) involvement in development and course of IBD, the lack of investigation of the relationship between themselves and with prostanoids necessitate further research in this direction. The study of ILs content in systemic blood flow and ILs relationship with other inflammatory mediators particularly prostanoids in colon tissue will make it possible to evaluate the disease course state and the treatment effect without invasive methods.

AIM

The aim is to study the IL-1β, IL-6, IL-8, IL-10 blood serum levels and their relationship with colon tissue prostanoids in experimental UC.

MATERIALS AND METHODS

The study used 42 adult WAG rats, divided into 3 equal groups. The first control group rats were rectally injected with sauline; the second control group rats were injected with a 50% ethanol solution; the experimental group rats were injected with DNBS in a 50% ethanol solution for 14 days [11]. On 15th day, the animals were removed from the experiment using a guillotine knife.

ELISA Kits for rats Interleukin 1Beta, IL-6, IL-8, IL-10 ("abcam", USA) were used to determine blood serum ILs levels. In colon homogenates, the prostanoid contents were determined by Rat PGE2, RAT PGF2α, RAT PGI2, RAT TXB2 ELISA Kits (MyBioSource) (USA) and 8-epi-PGF2α ELISA Kit (Elabscience) (USA) with tissue pretreatment according the kits instructions.

Statistical data processing was carried out using GraphPad Prism 5 Software (USA). Comparisons between two independent groups of variables were performed using non-parametric Mann–Whitney U test. Results are represented as medians and interquartile ranges. Correlation analysis according Spearman was used to reveal relationship between different variables of the same group. Differences were considered significant at $p<0.05$.

The study design was approved by the Commission of Ethics and Bioethics of Kharkiv National Medical University. The research was carried out in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes and the Council of Europe Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS123).

RESULTS

According to the obtained results IL-1β and IL-10 blood serum levels in rats with DNBS-induced UC were significantly higher versus 1st and 2nd controls. Both IL-1β and IL-10 were also increased in 2nd control versus 1st control. Blood serum levels of IL-6 and IL-8 in rats with DNBS-induced UC were significantly higher compared with 1st control but they didn't differ from 2nd control. Both IL-6 and IL-8 levels were enhanced in 2nd control versus 1st control (Table 1). In 1st control, positive correlations between IL-1β and IL-6 (r=+0.64, p=0.024), between IL-1β and IL-10 (r=+0.58, p=0.047), and a close negative relationship between IL-8 and IL-10 (r=-0.60, p=0.041) were revealed. In 2nd control, only positive relationship between IL-1β and IL-6 (r=+0.66, p=0.019) remained. In experimental group, positive relationship between IL-1β and IL-10 (r=+0.61, p=0.035), as well as negative relationship between IL-10 and IL -8 (r=-0.70, p=0.011) remained.

When studying the relationships between blood serum ILs and colon tissue prostanoids the following patterns were revealed. In 1st control group, there were negative correlations between PGF2α and all studied ILs, except IL-6, as well as close positive correlations between TXB2 and two ILs (IL-1β, IL-6). On the contrary, in 2nd control and experimental groups, PGF2α had close negative correlation only with IL-6. In addition to this, close positive correlations between IL-1β and PGI2, between TXB2 and 3 ILs (IL-1β, IL-8, IL-10) were found in 2nd control group. Even more relationships were found in experimental group. In this group, in addition to negative correlation between IL-6 and PGF2α, a negative correlation between IL-8 and 8-iso-PGF2α was also found. For 3 ILs (IL-1β, IL-6, IL-8), close positive associations with PGI2 were revealed. For all ILs, except IL-8, strong relationships with PGE2 were found (positive for IL-1β, IL-6; negative for IL-10). For 2 ILs (IL-1β and IL-8), close positive relationships with TXB2 were found (Table 2).

DISCUSSION

The development of various inflammatory processes, including colitis, largely depends on inflammatory mediators. They are numerous, produced by various cells, have pleiotropic and mutually modulating effects, that difficults the results interpretation to make the real role of each of them in the disease development. We investigated 4 ILs, three of which are mostly

Table 1. The interleukins levels (pg/ml) in blood serum of rats with experimental ulcerative colitis (Me [25%; 75%])

Interleukins	1st control group	2nd control group	Experimental group
IL-1β	9,67 [6,24; 14,30]	27,09 [20,43; 29,24]***	50,38 [38,07; 64,64]***, ΔΔΔ
IL-6	6,34 [5,29; 6,77]	25,32 [19,59; 25,91]***	25,66 [22,01; 26,97]***
IL-8	9,60 [8,56; 10,27]	32,31 [25,81; 41,05] ***	35,42 [30,86; 36,52] ***
IL-10	14,46[10,45; 16,63]	27,24[23,70; 31,73]***	42,87 [35,67; 48,79]***, ΔΔΔ

Note: *** - p < 0,001 versus 1st control group; ΔΔΔ - p<0.001 versus 2nd control group.

Table 2. The correlation coefficients between blood serum interleukins and colon tissue prostanoids in rats with experimental ulcerative colitis

Interleukins	Prostanoids				
	PGF2α	PGE2	PGI2	TXB2	8-epi-PGF2α
1st control group					
IL-1β	-0,61 *	+0,01	+0,08	+0,59*	+0,27
IL-6	+0,11	+0,04	-0,20	+0,64*	-0,55
IL-8	-0,59*	+0,17	+0,10	+0,05	-0,63
IL-10	-0,88***	-0,02	+0,10	+0,05	-0,63
2nd control group					
IL-1β	+0,03	+0,53	+0,65*	+0,75**	+0,47
IL-6	-0,66*	+0,56	+0,45	+0,33	-0,49
IL-8	+0,50	+0,03	+0,46	+0,86***	+0,49
IL-10	-0,50	+0,35	-0,15	+0,59*	-0,67
Experimental group					
IL-1β	-0,43	+0,70*	+0,63*	+0,65*	+0,18
IL-6	-0,69*	+0,82**	+0,71*	+0,57	-0,69
IL-8	-0,39	+0,55	+0,72**	+0,76**	-0,84*
IL-10	-0,56	-0,58*	+0,15	+0,32	-0,56

Note: * - p < 0,05; ** - p < 0,01; *** - p < 0,001.

considered proinflammatory (IL-1β, IL-6, IL-8) and one antiinflammatory and immunomodulatory (IL-10). We found an increase of all 4 ILs in serum of rats with DNBS-induced colitis versus 1st control, but only 2 of them (IL-1β, IL-10) were enhanced versus 2nd control.

In previous works, we showed, that content changing both isoforms of cyclooxygenases (COX-1 and COX-2) and content changes of most of investigated prostanoids in colon tissue are also due to ethanol, not DNBS [12, 13]. Ethanol is used in this experimental colitis model as intestinal barrier «destroyer». It destroys intestinal barrier by two ways: by epithelial cells disruption and spaces disruption between epithelial cells [14]. Ethanol induces intestinal inflamation not only by increasing intestinal mucosa permeability, but also by dysbiosis and mucosa immune system alterations [14].

The analysis of correlations between studied ILs indicates the certain relationships loss between ILs in 2nd control and experimental groups, which may be associated with macrophage phenotypes different ratio, namely the predominance of M1 phenotype in 2nd control group and M2 phenotype in experimental group. The microenvironment in which macrophage is located provides it with various signals that divergently shift the macrophage phenotype towards «classically activated» (M1) or «alternatively activated» (M2a, M2b or M2c) [15]. M2b and M2c phenotypes secrete high levels of IL-10, which in turn supressis production some proinflammatory factors, including IL-8 (Fiorentino DF et al., 1991) [15]. Namely the relationships between IL-10 and IL-6, as well as between IL-10 and IL-1β, were remained in experimental group. And the only relationship between IL-1β and IL-6 was remained in 2nd control group.

In the analysis of correlations between ILs and prostanoids, contrary to expectations, no correlations between the studied ILs and PGE2 were found in both control groups. Only in experimental group, positive correlation between PGE2 and all ILs, except IL-8, was observed. The existence of negative correlations between ILs and PGF2α and positive correlations between ILs and TXB2 was noteworthy. While in 1st control group all ILs, except IL-6, had negative

correlations with PGF2α, in the groups of rats exposed to either ethanol or DNBS in ethanol solution, these relationships were lost, but appeared a negative relationship between PGF2α and IL-6. In the available literature, we did not find works related to PGF2α role study in ulcerative colitis development, but in the work of Riaposova L. et al. (2023) it was shown that PGF2α enhanced inflammation in myometrial cells through increased activation of NF-κB and MAP kinases and increased COX-2 expression [16]. IL-6 is generally considered proinflammatory, but IL-6-/-/IL-10-/- mice were found to exhibit more pronounced intestinal inflammation and an earlier disease onset than IL-10-/- mice [17], that may indicate its antiinflammatory properties. This opinion is consistent with the work of Ding Q. et al. (2023), according to which bone marrow mesenchymal stem cells pretreated with IL-6 significantly reduced colon damage score in rats with DSS-induced UC [18].

As for TXB2, a positive relationship between this prostanoid and IL-1β was found in all groups. While positive correlation between TXB2 and IL-6 was also observed in the animals control group, in rats with inflammation provoked by both ethanol itself and ethanol dissolved DNBS, this relationship was lost, but there was relationship with IL-8. Only rats with ethanol-induced inflammation showed positive correlation between TXB2 and IL-10. TXA2 is the most abundant product of COX-1 arachidonic acid (AA) metabolism in mature human platelets (Ricciotti and FitzGerald, 2011) [19], while monocytes/macrophages synthesize TXA2 using COX-2 enzyme [20]. COX-1 participates in synthesis of TXA2 in B cells, and COX-1–derived TXA2 functions in an autocrine manner in developing B cells [21]. In the work of Sacco A. et al. (2019) was shown that specific deletion of COX-1 in platelets, i.e. inhibition of TXA2 production in platelets, resulted in milder symptoms of colitis, in exacerbation phase, and almost complete recovery from disease after withdrawal of DSS [19].

After calculating the correlation coefficients between blood serum ILs levels and colon tissue COX content, we found that only IL-8 had a close relationship with COX. This relationship was negative and observed in all 3 groups: in 1st control - between IL-8 and COX-2 (r=-0.60, p=0.039), in 2nd control and experimental groups - between IL- 8 and COX-1 (r=-0.79, p=0.002 and r=-0.63, p=0.046, respectively). The synthesis of all prostanoids, including PGE2, PGF2α, PGI2 and TXA2 (TXB2 is the stable metabolite of TXA2) is initiated by COX. It catalyzes AA conversion to PGH2. Futher PGH2 conversion to respective prostanoids is provided by other specific enzymes. We didn't found any correlation COX and prostanoids amounts, except negative correlation between COX-2 and TXB2 both in 2nd control and experimental groups (r=-0,61, p=0,036 and r=-0,65, p=0,033, respectively). The appearance of correlation between TXB2 and namely COX-2 in colonic tissue during inflammation may be related to TXB2-producing cells ratio change. The inconsistency between the correlations nature and TXB2 content may be related to changing in the functional and phenotypic properties of these cells and involwing the additional factors in regulation. The possibility of changing functional and phenotypic properties of cells, and thus regulation, as a result of pathology is confirmed in the work of Charalambous S. et al. [22]. According to the work, transforming growth factor-β1 down-regulates IL-8 production in normal brain endothelial cells (BEC), but do'nt inhibit IL-8 production in tumor-associated BEC [22]. One of the regulatory factors can be 8-epi-PGF2α, formed nonenzymatically as a result of increased lipid peroxidation. It has been shown to interact with TXA2 receptors [23], so it can perform TXA2-like effects including feed-back down-regulation of IL-8 production. This is evidenced by the significant negative relationship we found between IL-8 and 8-epi-PGF2α in experimental group.

The interpretation of data obtained is complicated by the fact that ILs can exert their effects through prostanoids, and prostanoids influence ILs release, as well as the fact that many types of cells are involved in the inflammation process and their ratio may be different in different phases of inflammation, and immunocompetent cells activation and cells response to signals depends on the microenvironment. Despite this, colon tissue prostanoids content changes are the result of the influence of all possible factors at once, including enzymes of synthesis and decay, the pool of receptors, interactions, microenvironment, etc. And the blood serum ILs content reflects the systemic processes in the body occuring in response to colon inflammation.

CONCLUSIONS

Ulcerative colitis in rats is accompanied by increase of all studied ILs in blood serum. The increase in all ILs level in 2nd control group compared to 1st control group, as well as the absence of a difference in the content of IL-6 and IL-8 between 2nd and experimental groups, are obviously conditioned with ability of ethanol to cause colon inflammation on its own.

The loss of certain relationships between ILs in the animals of 2nd control and experimental groups was found, that may be associated with a different ratio of macrophage phenotypes.

The existence of negative correlations between ILs and PGF2α, as well as the appearance of negative correlations between PGF2α and PGE2 in the groups of rats exposed to either ethanol or DNBS in ethanol solution, indicate the possible important role of PGF2α in the development of UC and the need for research in this direction.

Due to strong negative correlation between the blood serum IL-8 and the colon tissue COX-1 content in both ethanol- and DNBS-induced UC, increasing blood serum IL-8 level may be used as marker of reducing colon tissue COX-1 content in UC.

A significant negative correlation between 8-epi-PGF2α and IL-8 and a significant positive correlation between 8-epi-PGF2α and PGF2α, as well as the ability of 8-epi-PGF2α to bind to receptors to TXA2 testify to the possible role of 8-epi-PGF2α in UC development and justify the importance of research on the link of 8-epi-PGF2α, IL-8, PGF2α for understanding the mechanisms of this disease development and progression.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Iryna Vasylieva
Kharkiv National Medical University
4 Nauky avenue, 61022 Kharkiv, Ukraine
e-mail: vasilevaira@ukr.net

ORCID AND CONTRIBUTIONSHIP

Iryna Vasylieva: 0000-0002-4475-167X A B C D
Oksana Nakonechna: 0000-0002-2614-1587 A B C
Liudmyla Popova: 0000-0001-9967-8927 A D F
Natalia Yarmysh: 0000-0003-2798-4575 B
Anastasiia Bezrodna: 0000-0002-7543-7165 B
Nataliia Pustova: 0000-0002-1131-9725 E

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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