ORIGINAL ARTICLE

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Indoleamine-2,3-dioxygenase-related anti-inflammatory effects of 3-aminobenzamide and infliximab in experimental colitis

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SUMMARY

OBJECTIVE: This study aimed to investigate the presence of indoleamine-2,3-dioxygenase and bacterial translocation after the administration of 3-aminobenzamide and infliximab in the TNBS model of rat colitis.

METHODS: The study group was divided into five categories as follows: group 1: (control), group 2: colitis+saline, group 3: colitis+3-aminobenzamide, group 4: colitis+infliximab, and group 5: colitis+3-aminobenzamide+infliximab. Intestinal mesenteric cultures were incubated on specific agar media plates under aerobic and anaerobic conditions, bacterial translocation was evaluated and assessed as colony-forming units per gram of tissue. Colonic tissue samples were evaluated by Western blotting method to detect the presence of indoleamine-2,3-dioxygenase.

RESULTS: The results obtained were as follows: group 1: normal gut flora; group 2: eight of nine samples had bacterial translocation, of which six of them had positive indoleamine-2,3-dioxygenase protein; group 3: five of nine samples had bacterial translocation, of which seven of them had positive indoleamine-2,3-dioxygenase; group 4: three of nine samples had bacterial translocation, of which seven of them had positive indoleamine-2,3-dioxygenase; and group 5: only one sample had exact indoleamine-2,3-dioxygenase protein.

CONCLUSION: Altered expression of indoleamine-2,3-dioxygenase results in a lower bacterial translocation via infliximab compared with 3-aminobenzamide treatment. Combined treatments emphasized different approaches for the new molecules related to indoleamine-2,3-dioxygenase.

KEYWORDS: Inflammatory bowel disease. Ulcerative colitis. 3-Aminobenzamide. Infliximab. Indoleamine 2,3-dioxygenase.

INTRODUCTION

The drug 3-aminobenzamide (3-AB) is a pharmacological inhibitor of poly (ADP-ribose) polymerase-1 (PARP)[1]. Infliximab, another pharmacological agent, is a chimeric monoclonal antibody formed against tumor necrosis factor alfa (TNF-α)[2]. Although the etiology of inflammatory bowel disease (IBD) is still unknown, the pathophysiology is concentrated on the destructive activity of the reactive oxygen and nitrogen radicals and the excessive production of pro-inflammatory mediators[3].

Disruption of the intestinal homeostasis and tolerance toward the resident microbiota can be a major mechanism involved in the development of IBD. Depending on the response to luminal antigens, a controlled inflammation occurs and this is rapidly downregulated with the elimination of the pathogen. However, this balance is spoilt at IBD in favor of chronic inflammation[4]. Indoleamine-2,3-dioxygenase-1 (IDO, EC1.13.11.41), which catalyzes the first and limiting step of tryptophan catabolism, is thought to play a role in the control of intestinal inflammation; however, its role in the intestinal immunity has not been fully understood. However, in patients with IBD, the IDO enzyme was shown to be expressed above the normal from the biopsies of lesions[5,6]. IDO is also induced by IFN-γ, which is both natural and the strongest inducer at the adaptive immune response and, though still under discussion, by TNF-α[2,7].

In this study, we aimed to compare the protein expression of rate-limiting enzyme IDO of the kynurenine pathway and the differences in bacterial translocation (BT) in the TNBS

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model of rat colitis treated with both PARP enzyme inhibitor and the TNF-α receptor blocker infliximab.

METHODS

This study was verified by Ankara Training and Research Hospital Education, Planning, Coordination and Ethic Committee (AEAH.2007.0227) and performed in accordance with the National Institutes of Health guidelines for the care and handling of animals.

Study protocol

The male Wistar albino rats weighing between 250 and 300 g were collected from Ankara Training and Research Hospital (Ankara, Turkey). Rats were divided into five separate groups and kept in five separate cages with conventional housing system at 12-h light/dark cycles till the end of the study period. Male rats were randomly divided into five groups as follows: group 1: sham+saline (n=8), group 2: colitis+saline (n=9), group 3: colitis+3-AB (n=9), group 4: colitis+infliximab (n=9), and group 5: colitis+3-AB+infliximab (n=7). All rats were fed with standard food, water was ad libitum, and food was stopped 24 h before the rectal administration of TNBS or saline to the subjects. The colitis model was formed with a mixture of 0.8 ml of 5% (40 mg) TNBS (Sigma Chemical Co., St. Louis, MO, USA) and 0.4 ml of absolute ethanol according to previous literature[8]. To form colitis, 1.2 ml of TNBS–ethanol (GATA Biochemistry Laboratory, Ankara, Turkey) mixture was then given rectally; slowly administered by using polyethylene catheter 0.7 mm in diameter 8 cm forward into the anus; subjects were hold upside down for 30 s so that the substance spreads on the colon surface[9].

A dose of 10 mg/ml of 3-AB (Sigma, USA) and 10 mg/kg of infliximab (Sigma) was prepared to administrate to the treatment group and protected at +4°C until the experiment.

Group 1 and group 2 injected saline (1 ml., i.p. every 12 h), group 3 treated with 3-AB (10 mg/kg, i.p. every 12 h), group 4 received infliximab (10 mg/kg, i.p. every 24 h), and group 5 received both 3-AB and infliximab (3-AB 10 mg/kg, i.p. every 12 h, infliximab 10 mg/kg, i.p. every 24 h) 24 h before colitis was formed. A dose of 1.2 ml of saline at room temperature was rectally administered to group 1. After 7 days, rats were sacrificed under general anesthesia by cervical dislocation sacrification procedure. Colon was resected by laparotomy with a median incision in the supine position, in the manner to include healthy borders 2 cm to the proximal and distal to the segment with colitis in group 2 and group 3, which included approximately 6 cm segment of rectum. The part of the distal rectum included 6 cm segment of the rectum was removed in group 1. Macroscopically colon segments with mucosal focal hyperemia, ulceration, and thickening on colon wall were accepted as segment with colitis[10,11]. It was also sent to the pathological lab for microscopic examination and the next 3-cm colonic segment was placed into the cold chain for biochemical studies.

Those slides for histopathological examination were stained by hematoxylin and eosin (H&E). The total histological score was found by adding the epithelium and infiltration scores[12].

Tissue samples were tested daily by Western blotting method for the detection of IDO. Total protein concentration was measured by a commercial Pierce BCA Protein assay kit using bicinchoninic acid (BCA).

Mesenteric tissue complex was excised under sterile conditions, transferred into the test tubes containing thioglycolate broth medium (Merck, Germany), and cultured to investigate the BT.

Statistical analysis

Analysis of data was done with SPSS version 17 package program. Descriptive statistics were given as mean value±standard deviation. Differences in continuous variables and significance of the difference in terms of averages between groups were analyzed using the Mann-Whitney U test. Categorical variables were analyzed using chi-square tests. Results for $p<0.05$ were accepted statistically significant.

RESULTS

Histopathological finding among groups

The total scores of histopathology are shown in Figure 1. The pathological scores of groups 3 and 4 were statistically significantly decreased compared to group 2 ($p<0.05$).

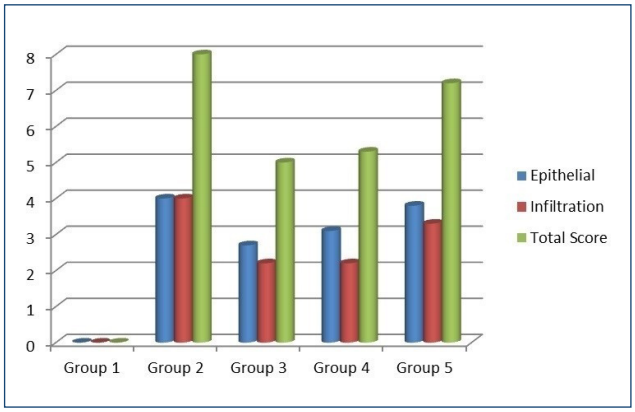

Figure 1. Evaluation of the histopathological findings.

Association between bacterial translocation and indoleamine-2,3-dioxygenase expression

The total bacterial amounts (cfu/ml) cultured under aerobic and anaerobic conditions are presented in Table 1.

The chemiluminescence images of polyvinylidene difluoride (PVDF) membranes belonging to β-actin and IDO protein bands in all groups were detected. The difference between the groups for the culture results and IDO expression was evaluated as percentage in order to apply the results easily for clinical interpretations. Overall, the culture results and IDO expression were detected in group 1 (normal gut flora), but BT and IDO were not exactly detectable in all samples. In group 2, 8 (88.9%) of the 9 samples had BT, of which 6 (66.7%) samples had positive IDO protein. In group 3, 5 (55.6%) of the 9 samples had BT, of which 7 (77.8%) samples had positive IDO protein. In group 4, 3 (33.3%) of the 9 samples had BT and IDO protein was positive in 7 (77.8%) of these samples. In group 5, no BT was found and none of the samples had exact IDO protein; only one might had IDO protein, which we considered positive. Finally, the relations between BT percentage (%) and IDO expression (%) are presented in Table 2.

DISCUSSION

This study investigated the effects of TNF-α and PARP inhibitors on IDO-induced BT depletion in an experimental IBD model. IDO expression, the major enzyme in tryptophan metabolism, was measured and the BT formation was evaluated in TNBS-induced model. The evaluation of damage in the colonic specimens was mainly done histologically, and the characterization of the intestine microbiota was performed by microbiological methods. While discussing the relationship of BT and IDO expression as percentage results, we observed either similar or contrary results to literature. Both treatments decreased the BT%, but the combination therapy was confusingly the best treatment for BT. The major results of the study were that BT was inhibited by using TNF-α and PARP inhibitors and the expression of IDO was lower in those groups. However, a remarkable decrease in IDO expression was observed in both 3-AB and infliximab combined treated group.

Although gut-associated microbial community reveals anaerobic and aerobic bacteria in healthy subjects, intestinal inflammation is associated with the disturbance of the microbiota and often includes an increased prevalence of

Table 1. Total bacterial amount (cfu/mL) cultured under aerobic and anaerobic conditions.

	Groups					p-value
	1	2	3	4	5	
	Mean±SD Median (min-max)	Mean±SD Median (min-max)	Mean±SD Median (min-max)	Mean±SD Median (min-max)	Mean±SD Median (min-max)	
Aerobic conditions (cfu/ml)	24.50±6.25 23.00 (15–35)	34.67±15.13 32.00 (13–60)	28.11±27.09 15.00 (6–80)	38.11±29.01 33.00 (7–86)	27.29±19.57 19.00 (4–60)	(0.615) NS
Anaerobic conditions (cfu/ml)	26.13±19.11 16.00 (11–58)	34.00±51.17 13.00 (2–154)	14.56±9.26 11.00 (4–34)	47.78±55.31 24.00 (3–171)	43.00±38.54 31.00 (6–120)	(0.242) NS
Total (cfu/ml)	50.63±24.80 38.50 (31–93)	68.56±56.12 47.00 (18–202)	43.78±32.15 40.00 (10–100)	85.56±69.28 61.00 (11–204)	70.29±49.61 39.00 (25–152)	(0.560) NS

Group 1: sham+saline (n=8), Group 2: colitis+saline (n=9), Group 3: colitis+3-AB (n=9), Group 4: colitis+infliximab (n=9), Group 5: colitis+ 3-AB+infliximab (n=7).

Table 2. Association between bacterial translocation (%) and indoleamine-2,3-dioxygenase expression (%) among groups.

		Groups (n, %)					p-value
		1	2	3	4	5	
BT	None	8 (100%)	1 (11.1%)	4 (44.4%)	6 (66.7%)	7 (100%)	<0.001
	Positive	0 (0.0%)	8 (88.9%)	5 (55.6%)	3 (33.3%)	0 (0.0%)	
IDO	None	8 (100%)	3 (33.3%)	2 (22.2%)	2 (22.2%)	6 (85.7%)	0.001
	Positive	0 (0.0%)	6 (66.7%)	7 (77.8%)	7 (77.8%)	1 (14.3%)	

Group 1: sham+saline (n=8), Group 2: colitis+saline (n=9), Group 3: colitis+3-AB (n=9), Group 4: colitis+infliximab (n=9), Group 5: colitis+ 3-AB+infliximab (n=7). BT: Bacterial translocation; IDO expression.

facultative anaerobic bacteria, which can further exacerbate inflammation[13]. Usage of anti-inflammatory and immunomodulatory agents can control mucosal inflammation and change clinical course of the disease by preventing complications[14]. During inflammation, one excepted idea is the electron acceptors generated as by-products of the host inflammatory response that can feed selectively facultative anaerobic bacteria. Also, excessive secretion of T-helper cells and chemokines can cause to degenerate tolerance and immunoregulation toward antigens by controlling T_{H1}-cell proliferation[15]. IDO facilitates the formation of suitable immune response by affecting the balance between immune tolerance and attack. It is also induced by TNF-α, IFN-α/β, and IL-10 which are produced during infections[16,17].

Generally, TNF-α, which is abundantly expressed from the intestines of IBD patients, is a pro-inflammatory cytokine that plays an important role in pathogenesis of IBD and contributes to enteritis as an induction of apoptosis in villous epithelial cells, disruption of the epithelial barrier, and secretion of chemokines in intestinal epithelial cells. Infliximab decreases CD25 expression and inhibits the release of IFN-γ, IL-13, IL-17A, and TNF in the CD4+ and CD8+ T-cell population[18]. In our study, 3-AB- and infliximab-treated groups had increased expression of IDO and reduced bacterial growth, which was due to an increased degradation of tryptophan. However, in infliximab-treated group, the expectation was the inhibition of TNF-α effect, but we observed 77.8% of IDO expression and had better results for BT%. The alterations in the enzyme activity by TNF-α inhibitor and PARP inhibitor might be used as an indicator in deciding whether to continue the pharmacotherapy and in determining the effect of drug. In the treated groups, the cause of an increased expression of IDO might be due to inadequate doses or inadequate routine treatment follow-up. Ciorba et al. suggested that increasing IDO expression within the intestine may have the therapeutic capacity to abrogate colitis[19]. In our study, IDO expressions were high in treatment groups 3 and 4 compared to colitis group and had lower histopathological scores with decreased BT%.

One of the main courses of IDO expression is to act as an effective agent preventing bacterial and/or viral infections of the closed-body fields such as ductus epididymis by decreasing the production of tryptophan[11]. BT is the transmission of vital endogen bacteria from intestinal lumen to mesenteric tissues and other gastrointestinal organs. There is a positive correlation between the severity of colitis and the BT. In a recent study, in group 2 (i.e., colitis group), a higher BT (88.9%) was observed. Both treatments could improve the BT%, but the combination of treatments was fully potent. The presence of IDO activity and depletion of local tryptophan can cause growth arrest of several tryptophan-dependent microorganisms as a local anti-infectious agent[20]. However, a conflicting result was found in group 5, with only one detectable IDO expression and nearly no BT, indicating that the synergistic effect of combined treatments might be efficient to yield the use of the enzyme to avoid BT. In another study, the inhibition of this enzyme worsen the disease severity, suggesting that it acts as a natural blocker in limiting colitis. In our study, we also had a higher score for histopathology, supporting the severity of disease in group 5.

One possible role of IDO in the endothelium may be as a free radical scavenger due to its use of superoxide anion. IDO may serve as an important antioxidant role[21]. Park et al. suggested that the IDO-mediated depletion of tryptophan and subsequent accumulation of active metabolites may have neuroprotective effects on ischemic injury to prevent hippocampal neuronal cell death[22].

CONCLUSIONS

Our study reports about the effects of TNF-α and PARP inhibitors on IDO-induced BT depletion in an experimental IBD model. The major results of the study reveal that BT was inhibited by using TNF-α and PARP inhibitors and the expression of IDO was decreased. This study can lead to the development of new molecules for pharmacological inhibition of IDO activity in several clinical settings. The detailed metabolic pathway studies for IDO will enable to design and develop new molecules for the pharmacological inhibition or the activation of IDO activity in several clinical settings.

AUTHORS' CONTRIBUTIONS

EM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft. **SA:** Data curation, Resources, Supervision, Visualization. **OUA:** Data curation, Resources, Software, Supervision. **DS:** Formal Analysis, Resources, Software, Supervision. **GA:** Resources, Software, Supervision. **AE:** Formal Analysis, Funding acquisition, Writing – review & editing. **ASD:** Conceptualization, Investigation, Methodology, Project administration, Validation, Writing – review & editing. **YO:** Conceptualization, Investigation, Methodology, Validation, Writing – review & editing. **MD:** Methodology, Validation, Writing – review & editing.

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