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Protective role of vitamin B12 on acetic acid induced colitis in rats

Şeyma Özsoy[1], Zeki Özsoy[2], Fikret Gevrek[3], Abdullah Özgür Yeniova[4]

[1] Department of Physiology, Tokat Gaziosmanpaşa University Faculty of Medicine, Tokat, Türkiye
[2] Department of General Surgery, Tokat Gaziosmanpaşa University Faculty of Medicine, Tokat, Türkiye
[3] Department of Histology, Tokat Gaziosmanpaşa University Faculty of Medicine, Tokat, Türkiye
[4] Department of Internal Medicine and Gastroenterology, Tokat Gaziosmanpaşa University Faculty of Medicine, Tokat, Türkiye

ABSTRACT

Objective: Inflammatory bowel disease (IBD) is a chronic, relapsing, and remittent inflammatory disease of the gastrointestinal tract. Nutritional deficiency may be instrumental in and attributable to this disease. We examined the effect of VitB12 supplementation on acetic acid (AA)-induced colitis in rats.

Material and Methods: Five minutes after the application of acetic acid to the rats to create a colitis model, VitB12 was administered 1 mg/kg, i.p concentration, then the application continued for three consecutive days. Control groups were included for colitis and VitB12. After 4d, the rats were sacrificed, and colonic tissues were harvested for macroscopic and microscopic examination of colonic damage. TNF-α, IL-1β, IL-6, MDA, GSH and SOD values were measured biochemically.

Results: There was statistically significant macroscopic improvement in damage to the colon tissues ($p< 0.05$). The severity of inflammation reduced in the VitB12 treated rat group compared with the control group, but was not significantly. The levels of TNF-α, IL-1β, MDA, and SOD did not differ between AA control and VitB12 treated AA colitis group. However, the levels of IL-6 and GSH were statistically significant different in rats with AA-induced colitis after VitB12 injection ($p< 0.05$).

Conclusion: Nutritional deficiencies might contribute to the pathogenesis of IBD, and the efficacy of VitB12 supplementation has controversial effects on the intestinal mucosa.

Keywords: Vitamin B12, inflammatory bowel disease, inflammation, acetic acid

INTRODUCTION

Inflammatory bowel disease (IBD), which has two major typical forms, ulcerative colitis (UC) and Crohn's disease (CD), is a chronic, relapsing and remitting inflammatory disease of the gastrointestinal (GI) tract. The relationship between individual genetic factors that regulate the innate and adaptive immune system, enteric microbiota, the enteric immune system, and environmental factors, particularly nutritional factors, are key determinants of IBD pathogenesis, and it has been suggested that these factors cause an excessive enteric immune response to gut flora or nutritional antigensis (1). Since multiple factors can contribute to inflammation of the intestinal mucosa, and nutritional deficiencies may occur due to malabsorption, inflammation, and resection, it has been postulated that nutritional deficiencies may actually evoke or influence colitis. The deficiencies in vitamin D, folate (vitamin B9), and VitB12 are among the most prevalent in IBD patients (2).

Vitamin B12 (VitB12), referred to as cobalamin, belongs to the B vitamin family. It is a critical coenzyme in various important metabolic processes that take place during the synthesis of nucleic acids, erythrocyte biogenesis, and the metabolism of amino acids and folate (3). Clinical medicine encounters VitB12 insufficiency frequently as a result of intestinal inflammation brought on by diarrhea, which impairs VitB12 absorption (4). For the diagnosis of VitB12 deficiency in healthy people, minimum serum values of 200 pg/mL have been established (5,6). Vitamin deficiencies in patients with IBD may cause clinical, biochemical, and inflammatory damage. For instance, anaemia in IBD has been linked to VitB12 deficiency (7).

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Corresponding Author
Şeyma Özsoy
E-mail: seyma.ozsoy@hotmail.com

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Additionally, a VitB12 deficiency prevents the pro-oxidant and pro-inflammatory homocysteine from being converted to methionine, causing homocysteine to build up in the blood and intestinal mucosa of IBD patients (8). As a result, VitB12 inadequacy is frequently present in patients with ileal dysfunction (2).

Deficiency of folate and VitB12 may be involved in the pathogenesis of IBD by modulating TNF-α mediated cytotoxicity and inducing the production of inflammatory cytokines and chemokines, such as monocyte chemoattractant protein (MCP-1) and IL-8 (9-11). Although several experimental and cohort studies have shown evidence of an association between the pathogenesis of IBD and VitB12 and folate deficiency (5,6), it is still not clear whether VitB12 deficiency leads to IBD. The aim of the present study was to assess the effect of VitB12 in acetic acid (AA)-induced colitis in rats. Additionally, our goal was to determine the protective role of VitB12 in colitis with biochemically inflammatory indices and markers of oxidative damage.

MATERIAL and METHODS

Animals

A total of 28 male Wistar-Albino rats weighing between 250 and 350 g were included, and were kept in separate cages in groups of two or three, at a constant temperature of 23°C and in a 12-hour light/dark cycle. They were fed a standard diet, and food and water were available ad libitum. All animals were maintained under fasting conditions for 24 h before undergoing surgical procedures, and no antibiotics were given before or after the procedures. Approval for the study protocol was granted by the Experimental Animals Ethics Committee of Gaziosmanpaşa University Medical Faculty. All experimental, surgical, and laboratory procedures were applied in Gaziosmanpaşa University Medical Faculty Experimental Research Centre and Gaziosmanpaşa University Medical Faculty Biochemistry and Histology Laboratories.

Induction of Acetic Acid (AA)-Colitis

The colitis model was induced by inserting a soft 6 mm pediatric-feeding catheter into the anus of each rat, and advancing the tip by 8 cm. One mL of 4% AA (pH 2.3) solution was slowly transrectally injected. In order to spread AA into the colon lumen, 2 mL of air was put into the catheter. Physical trauma was reduced by withdrawing the catheter slowly, and the rats were held upside down by the tail for 30 s to prevent any leakage of the administered substance. The experimental procedures were carried out under general anesthesia via an intramuscular administration of 75 mg/kg ketamine hydrochloride (Ketalar 500 mg flacon; Pfizer, İstanbul, Türkiye) and 10 mg/kg xylazine hydrochloride (Rompun 2% flacon; Bayer, İstanbul, Türkiye).

Experimental Group

The total 28 rats were randomized into four groups of seven. Group 1 (Control saline) was the control group, in which the rats received transrectal injections of saline; group 2 (AA colitis control) was the control colitis group, in which AA was administered into the colon of the rats; group 3 (VitB12) was the VitB12 treatment group, in which 1 mg/kg of VitB12 was intraperitoneally administered five minutes after saline injection, with treatment then continuing for three consecutive days; group 4 (VitB12 treatment in AA colitis) was the AA-induced colitis group in which 1 mg/kg VitB12 was intraperitoneally administered five minutes after colitis induction, with treatment then continuing for three consecutive days. All rats were sacrificed on day four after the induction of colitis by cervical decapitation (Figure 1). Before the procedure, 30 mg/kg hydrochloride and 5 mg/kg xylazine were administered as anesthesia. Intracardiac blood samples were drawn via making an incision of the abdomen and accessing the heart through the diaphragm. All blood samples were stored at -80°C until the day of analysis.

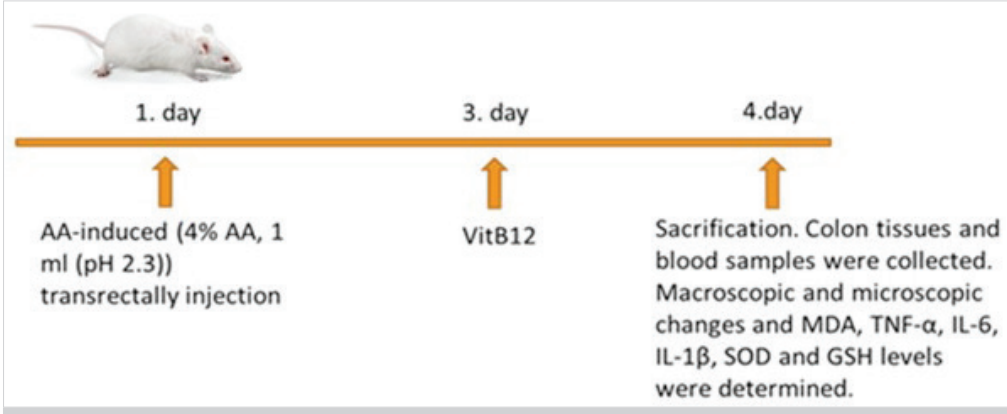

Figure 1. Experimental procedure.

Clinical Changes After AA Administration and Macroscopic Findings

Body weights were obtained before and after the colitis induction procedure. A new parameter, weight change (WC) was obtained according to the formula "body weight after the procedure-body weight before the procedure". Macroscopic assessment of the colon was made after the rats had been sacrificed. The longitudinally removed colon was opened and washed with saline. Mucosal lesions were then macroscopically scored, according to the Morris scoring system (12), as shown in Table 1.

Microscopic Changes

Colonic samples were chosen according to the Morris scoring system. The colon of each rat was macroscopically assessed, and samples of the region with the highest macroscopic score were obtained. These samples were then fixed in 10% buffer-neutral formaldehyde solution for 36 h. The fixed samples were embedded in paraffin, and 5 µm sections were obtained by cutting the paraffin blocks. Hematoxylin and eosin were used for staining after melting the paraffinized samples. Histologic assessment was carried out by a researcher blinded to the group information. A total of 10 sections from the seven rats in each group were considered, and an average of 20 microscopic views was assessed for each group. These views were analyzed via a computer-assisted light microscope (Nikon Eclipse 200, serial no: T1al 944909, Japan) with an integrated camera (Nikon Ds-Fi1, Japan), and transferred to the monitor for analysis using a Nis element program. Inflammation score (IS) was reported by the researcher, using a coding system that evaluates the intensity of inflammation in the colon strata. The four-level grading system is shown in Table 2 (13).

Biochemical Measurement

The blood samples were centrifuged at 4.000 rpm for 10 min at 4°C, and the removed plasma was stored at -80°C. Glutathione (GSH; item no: 7003002; Cayman Chemical, Estonia) and malondialdehyde (MDA; item no: 10009055; Cayman Chemical, Estonia) levels were measured with the colorimetric method, in accordance with the manufacturer's instructions, and superoxidismutase (SOD; cat no: YHB2870Hu;YH-Biosearch, China), TNF-a (cat no: YHB1098Ra; YH-Biosearch, China), interleukin (IL)-6 (cat no: YHB0630Ra; YH-Biosearch, China), and IL-1β (cat no:YHB0616Ra; YH-Biosearch, China) levels were measured using the enzyme-linked immunosorbent assay (ELISA) method.

Statistical Analysis

All statistical analyses were conducted by using SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as mean ± standard deviation and median (min-max). Normality distributions of the data were assessed by Shapiro-Wilk test. The significance of the difference between two paired groups was evaluated using Wilcoxon signed rank test. The significances of the difference between more than two groups were evaluated by using Kruskal-Wallis test (non-parametric analysis of variance) since data did not meet the assumptions of a parametric analysis of variance (ANOVA) test. Post-hoc test conducted after Kruskal-Wallis test in order to determine significant differences among the multiple groups with pairwise comparison. p value <0.05 was considered statistically significant.

Table 1. Macroscopic evaluation scale

Score	Criterion
0	No damage
1	Focal hyperemia without ulceration
2	Hyperemia or linear ulceration without thickening of colonic wall
3	Linear ulceration with inflammation in one area
4	Ulceration or inflammation in two or more areas
5	Two or more areas of major ulceration and inflammation, one or more sites of damage to the colon segment longer than 1 cm
6-10	The ulcer and inflammation area longer than 2 cm in the colon (the score is increased by 1 unit for every 1 cm damage)

Table 2. Inflammation grading scale

Score	Inflammation grade
0	No inflammatory cells
1	Giant cells, scattered few lymphocyte and plasma cell
2	Giant cells, increased amount of plasma cell, eosinophil, neutrophil
3	A great number of mixed inflammatory cells, microabscess formation

RESULTS

The Effect of VitB12 on AA-Induced Colitis

The body weights of the rats in all four groups were measured pre-experiment and post-experiment. The control saline group rats gained weight after saline injection, while the AA colitis control group rats lost weight following AA injection; the statistically difference was significant in both groups (p= 0.028; p= 0.018, respectively) (Table 3, Figure 2). There was no significant weight change in the other two groups. The new WC parameter was obtained by subtracting the post-experiment weight from the pre-experiment weight. A post-hoc analysis showed that the WC differences between the AA colitis control and control saline, AA colitis control and VitB12, control saline and VitB12 treatment in AA colitis groups were significant (p= 0.002, p= 0.027, and p= 0.03, respectively). There was no statistically difference between the AA colitis control and VitB12 treatment in AA colitis groups. Post-hoc analyses revealed significantly different mean macroscopic scores between the control saline group vs. the VitB12 treatment in AA colitis group and the control saline groups vs. the AA colitis control and VitB12 and AA colitis control groups (p= 0.032, p< 0.001, and p= 0.001, respectively) (Table 4, Figure 3). Post-hoc analysis showed mean

Table 3. Comparison of pre-post experiment weight and weight change means according to rat groups

			Pre-post weight changes			Pre-post weight differences			
		n	Mean ± SD	Median (min-max)	p	Mean ± SD	Median (min-max)	p	Post-hoc p
Control	Pre	7	277.86 ± 12.17	272 (265-300)	**0.028***	28.14 ± 25.07	24 (-4, 70)	**0.001****	**0.030[a]**
	Post	7	306.00 ± 34.33	294 (276-370)					
AA colitis	Pre	7	288.43 ± 35.86	270 (255-336)	**0.018***	-34.42 ± 20.65	-26 (-66, -13)		**0.002[b]**
	Post	7	254.00 ± 23.69	252 (232-302)					
VitB12	Pre	7	314.57 ± 48.60	308 (242-390)	0.176	14.57 ± 24.01	14 (-18, 48)		**0.027[c]**
	Post	7	329.14 ± 43.51	310 (290-388)					
VitB12 + AA colitis	Pre	7	352.86 ± 28.86	336 (324-392)	0.108	-18.00 ± 23.00	-22 (-46, 12)		
	Post	7	334.86 ± 32.30	336 (290-376)					

SD: Standard deviation, Min: minimum, Max: maximum.
*Statistically significant (p< 0.05), **Statistically significant (p< 0.01),
[a]Control - VitB12 + AA colitis, [b]Control - AA colitis, [c]AA colitis - VitB12.

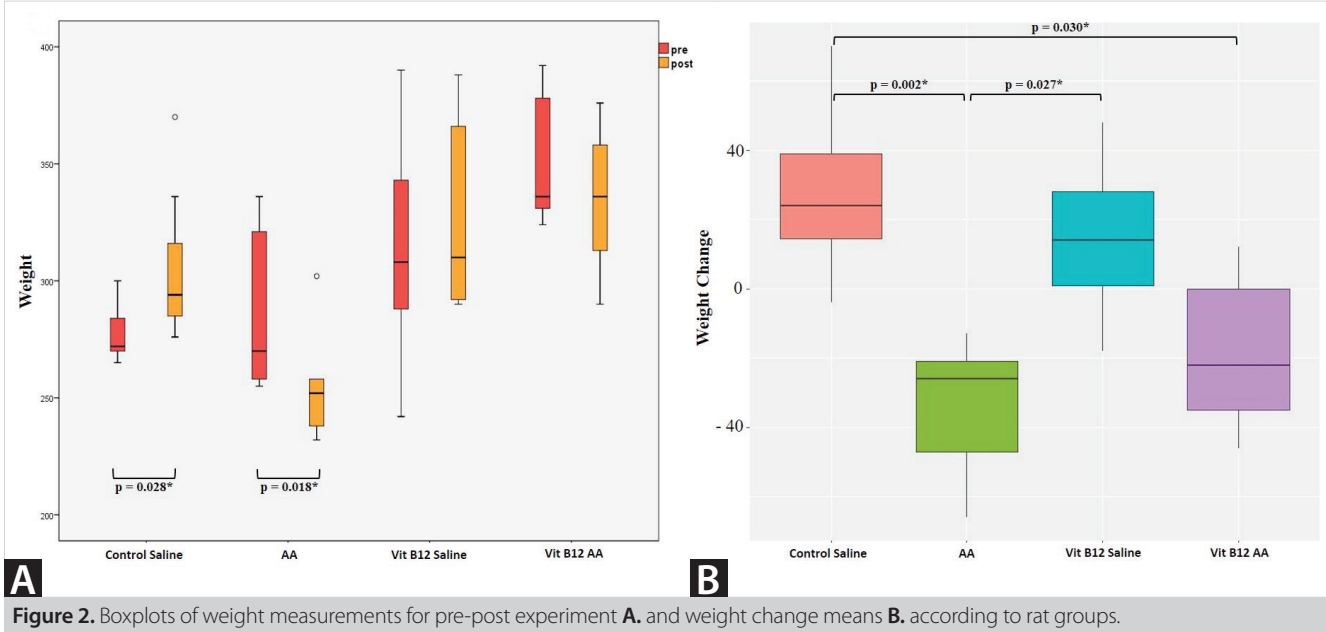

Figure 2. Boxplots of weight measurements for pre-post experiment **A.** and weight change means **B.** according to rat groups.

A. The control group and AA colitis group were significantly statistically different in pre-experimental and post-experimental weight loss (p= 0.028, p= 0.018, respectively). **B.** The control group was significantly statistically different when compared with the AA colitis and VitB12 + AA colitis groups (p= 0.002, p= 0.03, respectively). The AA colitis was significantly statistically different when compared with the VitB12 group (p= 0.027).

Table 4. Comparison of macroscopic damage score means according to rat groups

	n	Mean ± SD	Median (min-max)	p	Post-hoc p
Control	7	0.14 ± 0.37	0 (0-1)	<0.001*	**<0.001[a]**
AA colitis	7	6.57 ± 1.13	7 (5-8)		**0.001[b]**
VitB12	7	0.57 ± 0.53	1 (0-1)		
VitB12 + AA colitis	7	3.14 ± 0.90	3 (2-4)		**0.032[c]**

SD: Standard deviation, Min: Minimum, Max: Maximum.
*Statistically significant (p< 0.001).
[a]Control - AA colitis, [b]AA colitis - VitB12, [c]Control - VitB12 + AA colitis.

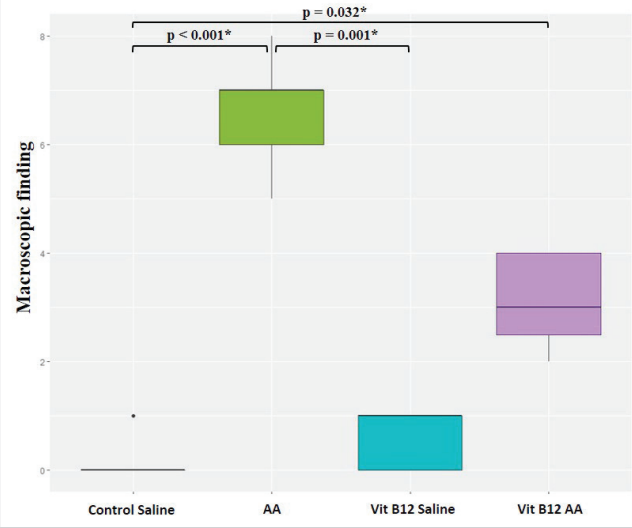

Figure 3. Boxplot for macroscopic finding according to rat groups. There were statistical differences between the control group vs. the VitB12 + AA colitis group and the control vs. the AA colitis groups, and the VitB12 and AA colitis groups (p= 0.032, p< 0.001, and p= 0.001, respectively).

differences in inflammation average between the VitB12 treatment in AA colitis and VitB12 groups, the AA colitis control and VitB12 groups, and the AA colitis control and control saline groups (p= 0.016, p< 0.001, and p= 0.005, respectively), as shown in Table 5 (Figure 4). Hematoxylin and eosin stained paraffin sections of four groups can be seen Figure 5. AA colitis control group has the greatest intensity of inflammatory cells. Control saline group appears to be normal colon tissue. The intensity of inflammation treatment decreased with VitB12 treatment in VitB12 and VitB12 treatment in AA colitis group but the only difference between VitB12 and AA colitis control is significant. There is no statistically significant difference between AA and VitB12 treatment in AA colitis groups.

Changes of Biochemical Measurement After VitB12 Administration in AA-Induced Colitis

All biochemical parameters and mean values are shown in Table 6. Different mean IL-1β values were observed between the VitB12 treatment in AA colitis and control saline groups, and the VitB12 treatment in AA colitis and VitB12 groups (p= 0.006, and p= 0.001, respectively), as shown in Table 6. Significantly different mean plasma IL-6 values were shown between the VitB12 treatment in AA colitis and control saline groups, the VitB12 treatment in AA colitis and the AA colitis control groups, and the VitB12 treatment in AA colitis and VitB12 groups (p= 0.048, p= 0.023, and p= 0.009, respectively). Significant differences in the mean serum TNF-α values between the VitB12 treatment in AA colitis and VitB12 groups and the control saline and VitB12 treatment in AA colitis groups (p= 0.025 and p= 0.021, respectively) were observed. There was a statistically significant difference between the VitB12 treatment in AA colitis and VitB12 groups (p= 0.001) in mean SOD values (Table 6, Figure 6). Post hoc analysis revealed a statistically significant difference in the mean GSH between the VitB12 treatment in AA colitis and VitB12 groups, and the VitB12 treatment in AA colitis and AA colitis control groups (p= 0.028 and p= 0.003, respectively). Significantly different mean serum MDA levels between the VitB12 treatment in AA colitis and VitB12 alone

Table 5. Comparison of inflammatory score means according to rat groups

	n	Mean ± SD	Median (min-max)	p	Post-hoc p
Control	7	0.32 ± 0.12	0.31 (0.21-0.57)	<0.001*	0.005[a]
AA colitis	7	2.02 ± 0.61	1.98 (1.17-2.86)		<0.001[b]
VitB12	7	0.26 ± 0.11	0.24 (0.14-0.48)		
VitB12 + AA colitis	7	1.31 ± 0.56	1.38 (0.70-2.24)		0.016[c]

SD: Standard deviation, Min: Minimum, Max: Maximum.
*Statistically significant (p< 0.001).
[a]Control - AA colitis, [b]AA colitis - VitB12, [c]VitB12 - VitB12 + AA colitis.

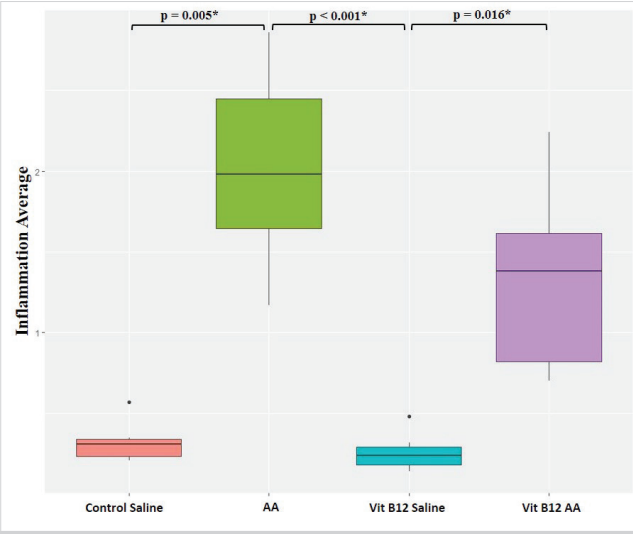

Figure 4. Boxplot for the inflammation score average according to rat groups. There were statistical differences between the control and AA colitis groups, the AA colitis and VitB12 groups, and the VitB12+AA colitis and VitB12 groups (p= 0.005, p< 0.001, and p= 0.016, respectively).

groups and the AA colitis control and VitB12 groups were observed (p= 0.002 and p= 0.010, respectively).

DISCUSSION

In the present study, we evaluated the effect of VitB12 administration on a rat model of AA-induced colitis under the hypothesis that VitB12 supplementation is essential in the prevention of IBD. As per results, VitB12 treatment in AA colitis group's values of IL-6, TNF- α, MDA, IL-1β, SOD, GSH were lower than AA colitis control group, but only IL-6 and GSH parameters reached the significant level. Furthermore, inflammation score and a macroscopic score of VitB12 treatment in AA colitis group were also lower than AA colitis control group. Few studies have investigated the association between VitB12 and colitis pathogenesis, and, to the best of our knowledge, the present study is the first and only experimental model that examined the effect of VitB12 supplementation modulating inflammation in a colitis rat model. Our results signified that although VitB12 can influence colitis, their influences seemed to be marginal and supplementary.

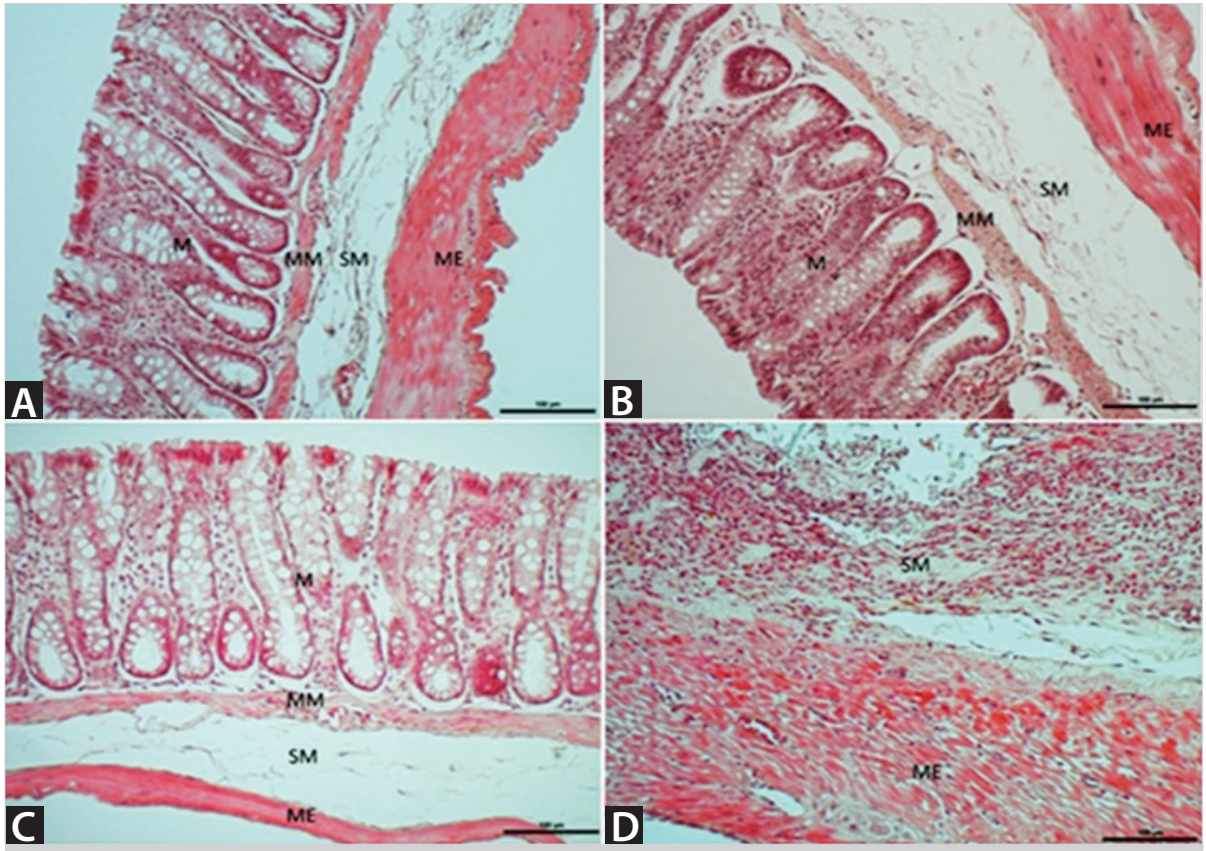

Figure 5. Photomicrographs of H&E stained paraffin sections of the colonic tissues of rats. **A.** Colon saline group showing normal mucosa with intact epithelial surface and normal tissue inflammatory cells. **B.** VitB12 + AA colitis group. **C.** VitB12 group seen to be generally similar to the control group. **D.** AA group showing destruction in all layers and severe crypt and MM damage, SM edema, significant epithelium loss, diffuse inflammatory cells infiltration and necrosis.
M: Mucosa, ME: Muscularis externa, MM: Muscularis mucosa, SM: Submucosa (hematoxylene and eosin, bar 100 µm).

Table 6. Mean comparisons for biochemical parameters

	G	N	Mean ± SD	Median (min-max)	p	Post-hoc p
IL-1β (pg/mL)	1	7	16.67 ± 0.96	16.73 (15.01-17.73)	**0.001****	**0.006**[a]
	2	7	16.24 ± 0.84	16.18 (15.26-17.35)		
	3	7	17.05 ± 0.46	17.05 (16.27-17.72)		**0.001**[b]
	4	7	12.83 ± 0.54	12.57 (12.40-13.96)		
IL-6 (ng/L)	1	7	224.66 ± 8.67	226 (210.66-233.33)	**0.005****	**0.048**[c]
	2	7	226.38 ± 8.26	226 (213.66-238.00)		**0.023**[d]
	3	7	227.14 ± 7.88	224 (217.66-237.00)		**0.009**[e]
	4	7	205.42 ± 9.98	201 (192.00-217.33)		
TNF α (ng/L)	1	7	209.65 ± 6.83	209 (199.33-218.33)	**0.011***	**0.021**[f]
	2	7	204.00 ± 12.64	195 (192.33-221.66)		
	3	7	209.09 ± 7.68	209 (199.00-219.00)		**0.025**[g]
	4	7	188.14 ± 10.40	186 (173.66-201.00)		
SOD (U/L)	1	7	624.86 ± 17.71	625 (604-647)	**0.002****	
	2	7	626.43 ± 27.38	627 (591-661)		
	3	7	642.29 ± 5.93	640 (634-651)		**0.001**[h]
	4	7	583.00 ± 23.55	587 (552-615)		
GSH (uM)	1	7	1.31 ± 0.28	1.25 (0.95-1.75)	**0.003****	
	2	7	1.56 ± 0.25	1.57 (1.29-2.02)		**0.003**[i]
	3	7	1.46 ± 0.49	1.32 (0.94-2.07)		**0.028**[k]
	4	7	0.72 ± 0.18	0.64 (0.57-1.01)		
MDA (uM)	1	7	3.63 ± 0.14	3.61 (3.44-3.85)	**0.002****	
	2	7	3.44 ± 0.33	3.41 (2.95-3.85)		**0.010**[l]
	3	7	4.31 ± 0.39	4.24 (3.84-4.85)		**0.002**[m]
	4	7	3.35 ± 0.34	3.34 (3.00-4.00)		

SD: Standard deviation, Min: Minimum, Max: Maximum.

*Statistically significant p< 0.05, **Statistically significant p< 0.01.

G: Groups (1= Control, 2= AA colitis, 3= VitB12, 4= VitB12 + AA colitis).

[a]Control - VitB12 + AA colitis, [b]VitB12 - VitB12 + AA colitis, [c]Control - VitB12 + AA colitis, [d]AA colitis - VitB12 + AA colitis, [e]VitB12 - VitB12 + AA colitis, [f]Control - VitB12 + AA colitis, [g]VitB12 - VitB12 + AA colitis, [h]VitB12 - VitB12 + AA colitis, [i]AA colitis - VitB12 + AA colitis, [k]VitB12 - VitB12 + AA colitis, [l]AA colitis - VitB12 , [m]VitB12 - VitB12 + AA colitis.

Clinical trials that aimed to find an association between the pathogenesis of IBD and vitamin B status have also been published, as they are observational prospective trials that investigated the effect of vitamin B on the course of IBD. As the studies that assessed the serum folate and VitB12 levels of patients with IBD have produced inconsistent results, meta-analyses have been conducted. The first meta-analysis, determined that people with CD have significantly higher levels of plasma homocysteine than do control groups. There was no difference between UC and CD patients (8). A recently published meta-analysis compared serum folate and VitB12 levels in people with IBD and healthy individuals. Interestingly, it found no difference in the mean of VitB12, but the folate levels did differ; people with UC patients had significantly lower serum folate levels than control group, but people with CD did not have different levels of folate from the control group (14).

Antioxidant mechanisms are essential for protecting the colonic mucosa from the harmful effect of inflammation (15). Increasing antioxidant defense mechanisms in the colon mucosa, via pharmacologic therapies, may be beneficial in IBD treatment. Excessive inflammatory response to oxidative stress exacerbates chronic inflammation including intestinal inflammation (16). The release of pro-inflammatory cytokines, such as IL-6, IL-1, and TNF-α, which are crucial to the start and progression of intestinal inflammation, is a major indicator of this disease (17). Padmanabhan et al. (2019) suggested that folate and VitB12 supplementation decreased the level of oxidative stress and ameliorated the cytotoxic effects of carsinogenesis in a rat

Figure 6. Boxplots for IL6 **A.** and TNF α **B.**, MDA **C.**, IL1β **D.**, SOD **E.**, GSH **F.** measurements according to rat groups.
A. The control group vs the VitB12 + AA group (p= 0.048); the AA group vs the VitB12+AA group (p= 0.023); the VitB12 group vs the VitB12 + AA colitis group (p= 0.009). **B.** The control group vs the VitB12 + AA colitis group (p= 0.021); the VitB12 group vs the VitB12+AA colitis group (p= 0.025). **C.** The AA group vs the VitB12 group (p= 0.010); the the VitB12 group vs the VitB12 + AA colitis group (p= 0.002). **D.** The control group vs the VitB12+AA group (p= 0.006); the VitB12 group vs the VitB12 + AA colitis group (p= 0.001). **E.** The VitB12 group vs the VitB12+AA colitis group (p= 0.001). **F.** The AA group vs the VitB12+AA group (p= 0.003); the VitB12 group vs the VitB12 + AA colitis (p= 0.028).

model of colon cancer (18). Moreover, it was demonstrated that the deficit in VitB12 and folate increased plasma level of IL-1β ve IL10 in rats subjected to dextran sodium sulphate (DSS) as an experimental colitis model (19). Similarly, VitB12-producing *Lactobacillus* (CLAB) attenuated colitis damage as well as inflammation and antioxidant markers [myeloperoxidase (MPO), malondialdehyde (MDA), interleukin 6 (IL-6), IL-1β and tumour necrosis factor-α (TNF-α)] in the mice colitis model formed with dextran sodium sulfate (DSS) (20). TNF-α mRNA levels and protein levels of p38, cytosolic phosphopolypase A2, and cyclooxygenase 2 were decreased in an experimental model of colitis induced by VitB12-treated DSS (21).

The present study investigates VitB12 as an antioxidant therapy for IBD, although many antioxidant compounds have yielded promising results as such treatment (22). Our study demonstrated that VitB12 supplementation decreased the levels of IL-6 and GSH in an AA-induced colitis rat model as similar to other studies. Although other biomarkers of inflammation (TNF-α and IL-1β) and oxidative stress (SOD and MDA) were reduced by VitB12, no significant results were observed. The experimental colitis models and the method and duration of administration of VitB12 to animals may differ. Evaluating specific markers of intestinal mucosa on an experimental colitis model that uses antioxidant supplementation may be more beneficial. Additionally, we used a standard diet, not a methyl-deficient diet.

According to Lurz et al. (2020), there were no appreciable variations in body weight between deficient and sufficient VitB12 diets in mice. Colon lengths in VitB12-deficient and VitB12-supplemented mice exposed to DSS were found to differ. In all treatment groups, an increase in the colonic expression of the anti-inflammatory cytokine IL-10 and in the pro-inflammatory cytokine TNF-α in post-DSS has been observed (23). Furthermore, a decrease in colonic tissue damage has previously been reported in the context of DSS. The increase in IL-10 is likely to be responsible for the reduction in tissue damage in VitB12-deficient mice (24). Similarly, in our study, IS and macroscopic score decreased in VitB12 treatment in AA colitis group. However, prior research has shown that VitB12 deficiency causes decreased populations of both CD8+ cell and NK cells, which could possibly account for the decrease in tissue-specific damage (25). Despite these results, multiple studies show that IBD patients have lower VitB12 levels than healthy controls (14). Although more research is required to completely understand the underlying mechanisms, the malabsorption of VitB12 in

colitis likely contributes to inflammation and tissue damage in the intestine.

CONCLUSION

We assessed the effect of VitB12 on AA-induced colitis and expected to see an anti-inflammatory benefit of giving VitB12. Our study demonstrated the benefit of VitB12 via the mean of inflammatory markers, such as IL-6, and the indirect oxidative stress marker GSH. These findings shed important light on the relationships between intestinal inflammation and VitB12. There are several limitations, however, that must be taken into account. The use of AA as a model of colitis is but one of many models of IBD (23). Local inflammatory and oxidative markers were limited in this study. Inflammation and oxidative markers in serum samples were analyzed, but these markers were not tested in colon tissue. Lastly, because only male rats were used, sex-related differences could not be distinguished. In conclusion, our results suggest that changing dietary VitB12 levels may affect conditions of intestinal health. The fact that the anti-inflammatory effect of VitB12 has been demonstrated in this study increases the scientific value of VitB12 for inflammatory diseases. If VitB12 is asserted as an anti-inflammatory, it will be valuable in terms of leading to new studies with vitamin B derivatives. Thus, delivering VitB12 supplements to IBD patients may enhance their healthier lives and prevent other illnesses.

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ORİJİNAL ÇALIŞMA-ÖZET
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Sıçanlarda asetik asit ile oluşturulmuş kolit üzerine vitamin B12'nin koruyucu etkisi

Şeyma Özsoy[1], Zeki Özsoy[2], Fikret Gevrek[3], Abdullah Özgür Yeniova[4]

[1] Tokat Gaziosmanpaşa Üniversitesi Tıp Fakülyesi, Fizyoloji Anabilim Dalı, Tokat, Türkiye
[2] Tokat Gaziosmanpaşa Üniversitesi Tıp Fakülyesi, Genel Cerrahi Anabilim Dalı, Tokat, Türkiye
[3] Tokat Gaziosmanpaşa Üniversitesi Tıp Fakülyesi, Histoloji Anabilim Dalı, Tokat, Türkiye
[4] Tokat Gaziosmanpaşa Üniversitesi Tıp Fakülyesi, Dahiliye ve Gastroenteroloji Anabilim Dalı, Tokat, TÜrkiye

ÖZET

Giriş ve Amaç: Enflamatuvar bağırsak hastalığı (IBD), gastrointestinal sistemin kronik, tekrarlayıcı ve remitan enflamatuvar bir hastalığıdır. Beslenme eksikliği bu hastalıkta katkıda bulunabilir. Biz çalışmamızda, VitB12 takviyesinin sıçanlarda asetik asit (AA) kaynaklı kolit üzerindeki etkisini incelemeyi amaçladık.

Gereç ve Yöntem: Kolit modeli oluşturmak için sıçanlara asetik asit uygulamasından beş dakika sonra VitB12 1 mg/kg, i.p üç gün süreyle uygulandı. Kolit ve VitB12 için kontrol grupları dahil edildi. Dört gün sonra, sıçanlar sakrifiye edildi ve kolon hasarının makroskopik ve mikroskobik incelemesi için kolon dokuları toplandı. Sıçanlardan alınan kan örneklerinde TNF-α, IL-1β, IL-6, MDA, GSH ve SOD değerleri biyokimyasal olarak ölçüldü.

Bulgular: Kolon dokularında iyileşme makroskopik olarak istatistiksel olarak anlamlıydı (p< 0,05). Kontrol grubu ile karşılaştırıldığında VitB12 ile tedavi edilen sıçan grubunda enflamasyonun şiddeti azaldı. AA ile indüklenmiş kolitli grupta VitB12 ile tedavi sonucu TNF-α, IL-1β, MDA ve SOD seviyeleri azalmıştı, fakat istatistiksel olarak anlamlı farklılık görülmedi. Ancak, B12 vitamini enjeksiyonundan sonra AA ile indüklenen kolitli sıçanlarda IL-6 ve GSH seviyeleri önemli ölçüde anlamlı azaldı (p< 0,05).

Sonuç: Beslenme eksiklikleri IBD patogenezine katkıda bulunabilir ve VitB12 takviyesi bağırsak mukozası üzerinde yararlı etkilere sahiptir.

Anahtar Kelimeler: Vitamin B12, enflamatuvar bağırsak hastalığı, enflamasyon, asetik asit

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