

Effect of Vitamin B12 on Acetic Acid Induced Colitis

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Research Article

Keywords: Vitamin B12, Inflammatory Bowel Disease, inflammation, intestine, acetic acid

Posted Date: March 10th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-237181/v1>

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Abstract

Background/Aims:

Inflammatory bowel disease (IBD) is a chronic, relapsing, and remittent inflammatory disease of the gastrointestinal tract. Nutritional deficiency may be instrumental and contributable in this disease, among which vitamin B12 deficiency has been identified in IBD. Since the relationship between vitamin B12 and IBD remains controversial, in this study, we have examined the effect of vitamin B12 supplementation on acetic acid (AA)-induced colitis in rats.

Methods

Total of 28 rats were randomized into four groups of seven animals per group; Group 1 (Control saline) was the control group, group 2 (AA colitis control) was the disease control group, group 3 (VitB12) was the vitamin B12 alone treatment group, group 4 (VitB12 treatment in AA colitis) was the AA-induced colitis VitB12 treatment group. Weight change was measured. Macroscopic and microscopic scores were measured in each group. Tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , IL-6, myeloperoxidase (MPO), malondialdehyde (MDA), glutathione reduced (GSH), and superoxide dismutase (SOD) were measured in each group.

Results

Macroscopic scores, as well as microscopic scores, were not different between the group. Also, the levels of TNF- α , IL-1 β , MPO, MDA, and SOD did not differ between AA control and VitB12 treated AA colitic group. However, the levels of IL-6 and GSH were significantly different in rats with AA-induced colitis after vitamin B12 injection.

Conclusion

Nutritional deficiencies might contribute to the pathogenesis of IBD, but in this study, the efficacy of vitamin B12 supplementation has controversial effects on the intestinal mucosa.

Introduction

Inflammatory bowel disease (IBD), including 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 an excessive enteric immune response to gut flora or nutritional antigens involved [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.

For instance, as far as nutritional factor is concerned, there is a lower incidence of IBD in the west of Canada, where more fresh fruit and vegetables are consumed than in the east of Canada, where fewer of these foods are consumed [2]. Furthermore, IBD is traditionally higher in developed countries where the consumption of fresh vegetables and fruit is lower than in developing countries. Deficiency of folate and vitamin B12 may be involved in the pathogenesis of IBD, and the consequence of such a deficiency is hyperhomocysteinemia [3]. Homocysteine may aggravate inflammation of the intestinal mucosa via an upregulation of proinflammatory genes. Previous studies have shown that homocysteine modulates TNF- α mediated cytotoxicity [4], in addition to inducing the production of inflammatory cytokines and chemokines, such as monocyte chemoattractant protein (MCP-1) and IL-8 [5]. Furthermore, oxidative stress, another determinant of IBD pathogenesis, is provoked in hyperhomocysteinemia. Excess homocysteine is reduced by oxygen radicals and by free sulfhydryl groups that can damage DNA, lipids, and proteins, and may cause mutations [6]. SOD activity is increased in hyperhomocysteinemia [7]. Folate produces epigenetic changes and affects the interaction between gut microbiota and the enteric immune system [8].

Although experimental and cohort studies have shown evidence of an association between the pathogenesis of IBD and vitamin B12 and folate deficiency, the evidence regarding the plasma homocysteine levels of IBD patients vs. those of controls is conflicting [9], as is that relating to the comparison of vitamin B12 and folate levels in IBD patients vs. controls [10]. In this background, the present study aimed to examine the effect of vitamin B12 administration on acetic acid (AA)-induced colitis in rats.

Materials And Methods

Rat 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 Gaziosmanpasa University Medical Faculty (2014-HAYDEK-29). All experimental, surgical, and laboratory procedures were applied in Gaziosmanpasa University Medical Faculty Experimental Research Centre and Gaziosmanpasa 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, Istanbul, Turkey) and 10 mg/kg xylazine hydrochloride (Rompun 2% flacon; Bayer, Istanbul, Turkey).

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 alone) was the vitamin B treatment group, in which 1 mg/kg of vitamin B12 was intraperitoneally administered 5 minutes after saline injection, with treatment then continuing for 3 consecutive days; group 4 (VitB12 treatment in AA colitis) was the AA-induced colitis group in which 1 mg/kg vitamin B12 was intraperitoneally administered 5 minutes after colitis induction, with treatment then continuing for 3 consecutive days. All rats were sacrificed on day 4 after the induction of colitis by cervical decapitation. Before the procedure, 30 mg/kg hydrochloride and 5mg/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.

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 [11], as shown in Table I.

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: T1a1

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 II [12].

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 myeloperoxidase (MPO; SEA601Ra; Cloud-CloneCorp, Katy, TX), 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-1 β (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 \pm 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.

Results

The effect of vitamin B12 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 difference was significant in both groups ($p = 0.028$; $p = 0.018$, respectively) (Table III, Fig. 1). 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 alone, 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 difference between the AA colitis control and VitB12 treatment in AA colitis groups (Table III, Fig. 1). 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 alone and AA colitis control

groups ($p = 0.032$, $p < 0.001$, and $p = 0.001$, respectively) (Table IV, Fig. 2). Post hoc analysis showed mean differences in IS between the VitB12 treatment in AA colitis and VitB12 alone groups, the AA colitis control and VitB12 alone 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 V (Fig. 3). Hematoxylin and eosin stained paraffin sections of four groups can be seen Fig. 4. 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 alone and VitB12 treatment in AA colitis group but the only difference between VitB12 alone and AA colitis control is significant. There is no significant difference between AA and VitB12 treatment in AA colitis groups.

Changes of biochemical measurement after vitamin B12 administration in AA-induced colitis

All biochemical parameters and mean values are shown in Table VI. There was no difference between groups in the mean of MPO ($p = 0.095$) (Table VI, Fig. 5). Different mean IL-1 β values were observed between the VitB12 treatment in AA colitis and control saline groups, and the VitB12 treatment in AA colitis and VitB12 alone groups ($p = 0.006$, and $p = 0.001$, respectively), as shown in Table VI. 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 alone groups ($p = 0.048$, $p = 0.023$, and $p = 0.009$, respectively), as shown in Table VI. Significant differences in the mean serum TNF- α values between the VitB12 treatment in AA colitis and VitB12 alone groups and the control saline and VitB12 treatment in AA colitis groups ($p = 0.025$ and $p = 0.021$, respectively) were observed, as shown in Table VI. There was a significant difference between the VitB12 treatment in AA colitis and VitB12 alone groups ($p = 0.001$) in mean SOD values (Table VI). Post hoc analysis revealed a significant difference in the mean GSH between the VitB12 treatment in AA colitis and VitB12 alone groups, and the VitB12 treatment in AA colitis and AA colitis control groups ($p = 0.028$ and $p = 0.003$, respectively, as shown in Table VI). Significantly different mean serum MDA levels between the VitB12 treatment in AA colitis and VitB12 alone groups and the AA colitis control and VitB12 alone groups were observed ($p = 0.002$ and $p = 0.010$, respectively), as shown in Table VI.

Discussion

In the present study, we evaluated the effect of vitamin B12 administration on a rat model of AA-induced colitis under the hypothesis that vitamin B12 supplementation is essential in the prevention of IBD. As results, VitB12 treatment in AA colitis group's values of IL-6, TNF- α , MDA, IL-1 β , 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, but the difference was not statistically significant. Few studies have investigated the association between vitamin B12 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 vitamin B12 supplementation modulates inflammation in a colitis rat model. Our results signified that although vitamin B12 can influence colitis, their influences seemed to marginal and supplementary.

Clinical trials that aimed to find an association between the pathogenesis of IBD and vitamin B status have also been published, as has an observational prospective trial that investigated the effect of Vitamin B on the course of IBD. The prevalence of hyperhomocysteinemia and the effect of hyperhomocysteinemia on disease activity were assessed via longitudinal follow-up of patients with UC and CD, there was no association between homocysteine levels and disease activity. Instead, the prevalence of hyperhomocysteinemia was higher than in a normal population, although there was no control arm [13]. Retrospective studies have reported a higher prevalence of hyperhomocysteinemia. Erzin et al. [14] reported that 56% of IBD patients, and Peyrin-Biroulet et al. [15] stated that 26% of CD patients have elevated homocysteine levels. Hyperhomocysteinemia can be a result of impaired vitamin absorption due to inflammation, deficiency resulting from a poor diet, and due to a resected or diseased ileum in the CD. Another retrospective study [16] revealed that people with CD had a higher prevalence of vitamin B12 deficiency than did those with UC and a control group. People with prior ileal or ileocolic resection have a particularly higher prevalence. There was no difference between the UC and control groups in this study, a finding that may be interpreted as vitamin B12 deficiency being a result of resection rather than intestinal inflammation. Another study reiterated these results. In a Swiss cohort, only CD patients with stenosis or intestinal surgery had vitamin B12 deficiency [16].

As the studies that assessed the serum folate and vitamin B12 levels of people with IBD patients 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 controls. There was no difference between UC and CD patients [17]. A recently published meta-analysis compared serum folate and vitamin B12 levels in people with IBD and healthy individuals. Interestingly, it found no difference in the mean of vitamin B12, but the folate levels did differ; people with UC patients had significantly lower serum folate levels than controls, but people with CD did not have different levels of folate from the control group [10]. The conflicting results may be due to the methodology used; one of these meta-analyses measured plasma homocysteine levels, while the other considered vitamin B12 and folate levels.

Although resection due to CD or intestinal inflammation can alter the absorption of vitamin B12 or folate, IBD can be a consequence of nutritional deficiency. Homocysteine is a sulfur-containing amino acid that plays a role in two major pathways: remethylation to methionine, which requires folate and vitamin B12, and transsulfuration to cystathionine, which requires vitamin B6. Irrespective of whether it is due to a methyl-deficient diet vitamin B deficient diet, or to genetic defects of enzymes that are involved in homocysteine metabolism (e.g., methyl tetra hydro folatereductase), hyperhomocysteinemia is known as one of the etiologic factors of thrombotic events [18]. It causes vascular inflammation via several mechanisms, one of which is increased production of reactive oxygen species. Similar activation takes place in the intestinal mucosa. Hyperhomocysteinemia promotes microvascular inflammation with

aggravating endothelial inflammation, resulting in vascular cell adhesion molecule – 1 upregulation, MCP-1 production, and p38 phosphorylation [19]. This is thought to promote mucosal inflammation and activate several inflammatory and oxidative stress pathways [20].

Antioxidant mechanisms are essential for protecting the colonic mucosa from the harmful effect of inflammation [21]. Increasing antioxidant defense mechanisms in the colon mucosa, via pharmacologic therapies, may be beneficial in IBD treatment. The present study is the first to investigate vitamin B12 as an antioxidant therapy for IBD, although many antioxidant compounds have yielded promising results as such treatment [22]. We expected that vitamin B12 supplementation will decrease the level of homocysteine that modulates inflammation on intestinal mucosa. Our study demonstrated that vitamin B12 supplementation decrease level of IL-6 and GSH in an AA-induced colitis rat model. However, the same results are not observed with all inflammation and oxidative stress biomarkers. Furthermore IS and macroscopic score difference between AA colitis control and VitB12 treatment in AA colitis group were not significant. There are not experimental colitis models that used vitamin B12 supplementation as an antioxidant therapy which we can compare with the present study. One experimental colitis model demonstrated that folate supplementation downregulated homocysteine-induced IL-17 and ROR- γ t expression in dextran sulfate sodium (DSS) induced colitis rats that were fed a methionine-deficient diet [23]. Homocysteine activated the p38/cPLA2/COX2/PGE2 pathway and also increased the expression of IL-17 and retinoid-related orphan nuclear receptor- γ t (ROR- γ t), which is the key transcription factor of IL-17. T helper 17 (Th17) cells, which derive from CD4⁺ T-cells, play an important role, and an imbalance between T regulatory cells and Th17 appears to be critical for the development of IBD [24, 25]. Although many cytokines play a role in IBD pathogenesis, some studies have suggested that IL-17, which is the cytokine of Th17, contributes to pathogenesis [26]. The present study evaluated the values of nonspecific inflammatory markers such as IL-6, IL-1 β etc. Maybe evaluating specific markers of intestinal mucosa such as IL-17 on an experimental colitis model that use antioxidant supplementation will be more beneficial. Furthermore, we used standard diet, not a methyl-deficient diet.

One experimental study on colitis evaluated the role of vitamin B on colitis sequela. In this study, we used dietary vitamin B deficiency and then created an experimental colitis model with DSS infusion. The disease progression and severity were determined by macroscopic changes and inflammation markers. Furthermore, this study determined the in vivo kinetics of the methionine pathway by measuring vitamin B6, vitamin B12, homocysteine and other metabolite levels in colon, plasma, and liver of mice. Interestingly, mortality rates, disease activity index and inflammation markers of mice group that were fed on a control diet were higher when compared with the mice that were fed on a vitamin B deficient diet. This study showed that homocysteine levels were higher in the plasma and colon of the deficient diet mice groups regardless of DSS status. Furthermore, vitamin B6 levels were determined indirectly by calculating its active form of pyridoxalphosphate (PLP). PLP levels were higher in the deficient diet mice groups. This study measured B12 associated metabolites to assess Vitamin B12 deficiency. Methylmalonic acid (MMA) is converted by an enzymatic reaction and requires VitB12 as a cofactor. MMA levels were higher in plasma when compared between the deficient diet mice and the control diet

mice groups. Further, this study indicated that remethylation and glycine levels were increased in the deficient mice group. These results indicated that the diet can overcome VitB6 deficiency but not that of VitB12. Folate was included in the diet so homocysteine level was increased due to the deficiency of vitamin B6 and not vitamin B12. Furthermore, this increase did not contribute to the disease progression or inflammation in DSS induced colitis model [27].

Experimental rodent IBD model (IL-10 knockout mice) was used and the effects of vitamin B6 inadequacy and supplementation were monitored on the severity of intestinal inflammation. Mice were randomly assigned to vitamin B6 deficient, replete, and supplementation group. The deficient and supplementation groups both had lower concentrations of molecular and histological markers of inflammation of colon than the replete group. The lower concentrations of histologic and molecular deficiency markers were in accordance with the study conducted by Benignt et al.; however, this study showed that vitamin B6 supplementation ameliorates intestinal inflammation [28]. The results reported by Benignt et al. and us are against the hypothesis of homocysteine contribution due to the nutritional deficiency of colitis pathogenesis and a decrease in its level with nutritional supplementation. Gut microbiota is associated with the pathogenesis of IBD. VitB12 producing bacteria may increase in case of acute intestinal inflammation and intestinal mucosa may absorb more VitB12 but supplementation of VitB12 may not ameliorate intestinal inflammation. We did not measure the level of VitB12 or its metabolites in colon and plasma. Furthermore, acetic acid-induced colitis model represents acute colitis rather than a chronic relapsing and remittent condition so we did not know the effect of vitamin B12 on a chronic colitis model. The effect of vitamin B6 deficiency and supplementation is associated with colitis rather than VitB12.

Conclusion

We assessed the effect of vitamin B12 on AA-induced colitis and expected to see an anti-inflammatory benefit of giving vitamin B12 as a drug. Our study demonstrated the benefit of vitamin B12 via the mean of inflammatory markers, such as IL-6, and the indirect oxidative stress marker GSH, but other biomarkers did not change. This may be explained by the fact that a methyl or vitamin B12 or/and B6 deficiency in the diet can exert a greater influence on inflammation than taking vitamin B12. This nutritional deficiency can influence intestinal inflammation more than taking extra antioxidants. Moreover, trials that use more specific markers for intestinal inflammation, such as IL-17, may be more informative in this research field. In conclusion, the use of vitamin B compounds to provide an anti-inflammatory effect in colitis requires further research in animal models and in clinical trials.

Declarations

Authorship Policy:

Seyma ve Zeki Ozsoy contributed to the study concept and design and the data acquisition. Abdullah Özgür Yeniova contributed to the statistical analysis and data analysis and interpretation. Fikret Gevrek contributed to the technical or material support.

CONFLICT OF INTEREST

Authors declared no conflict of interest

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Tables

Due to technical limitations, the tables are only available as a download in the supplemental files section.

Figures

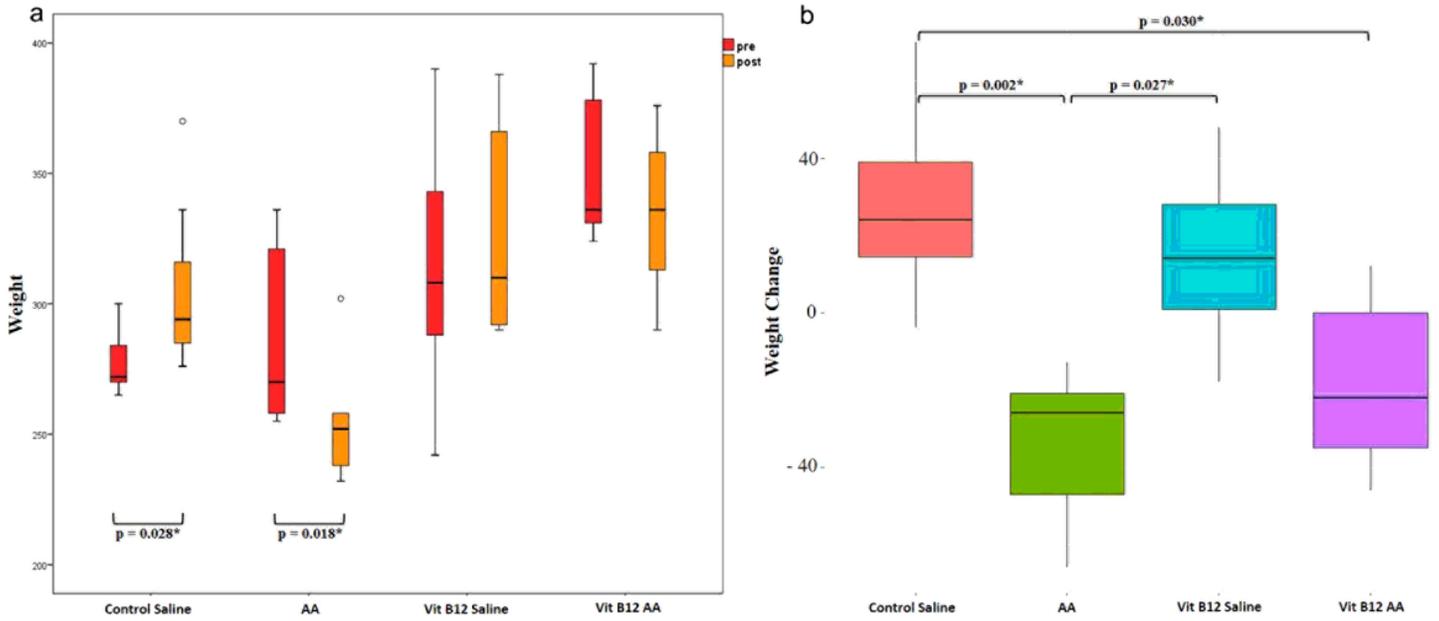


Figure 1

Boxplots of weight measurements for pre-post experiment (a) and weight change means (b) according to rat groups.

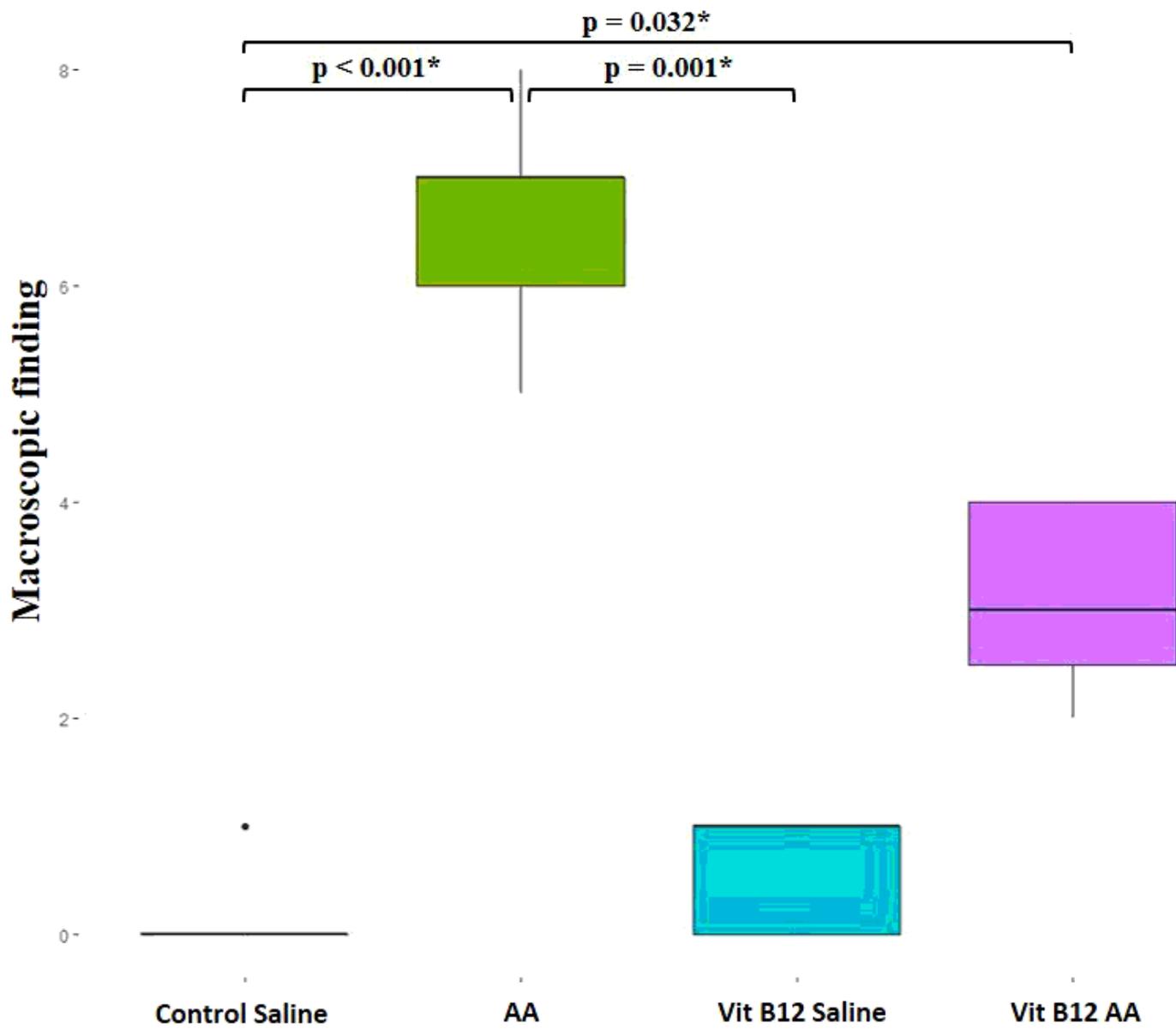


Figure 2

Photomicrographs of H&E stained paraffin sections of the colonic tissues of rats.

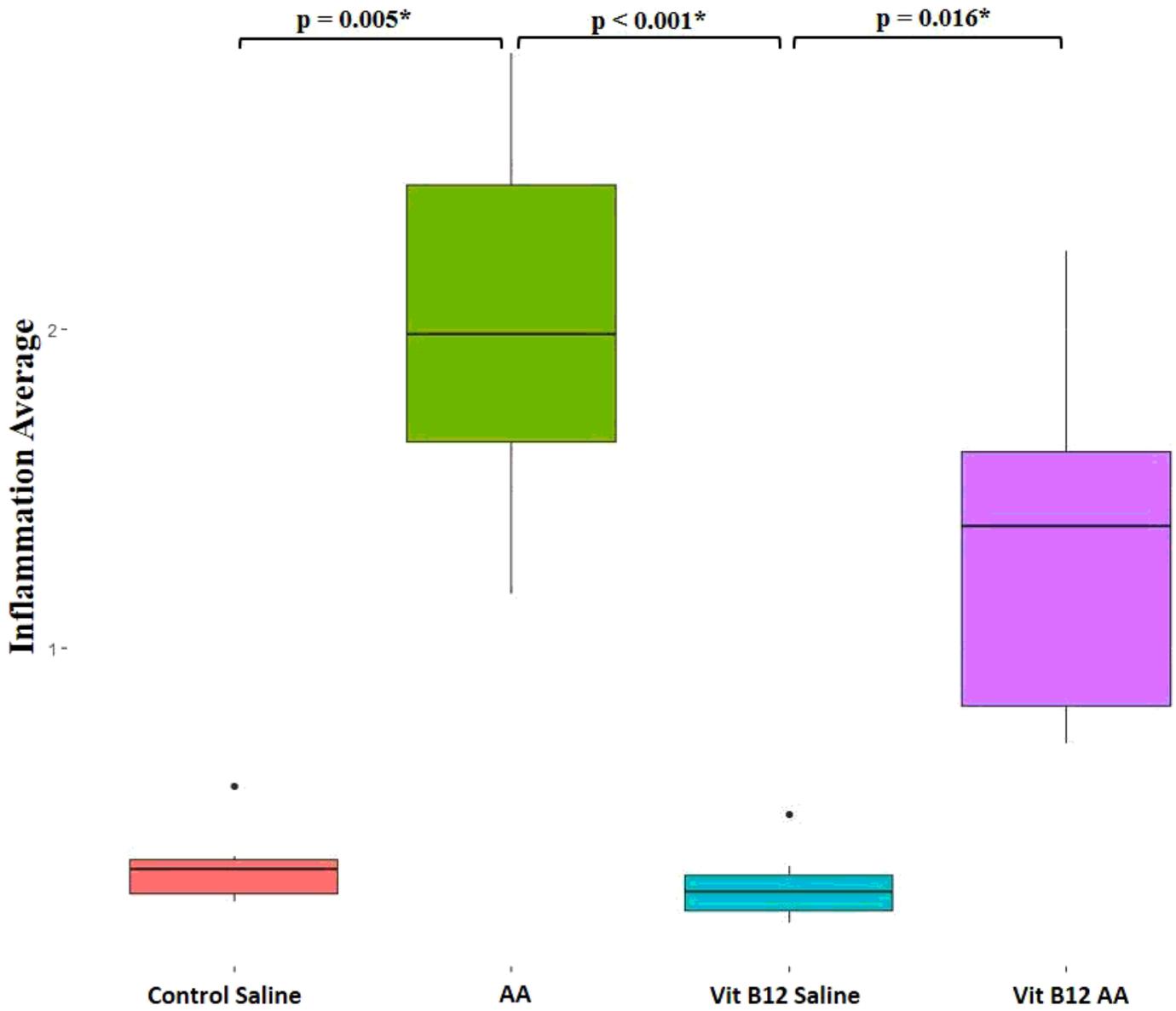
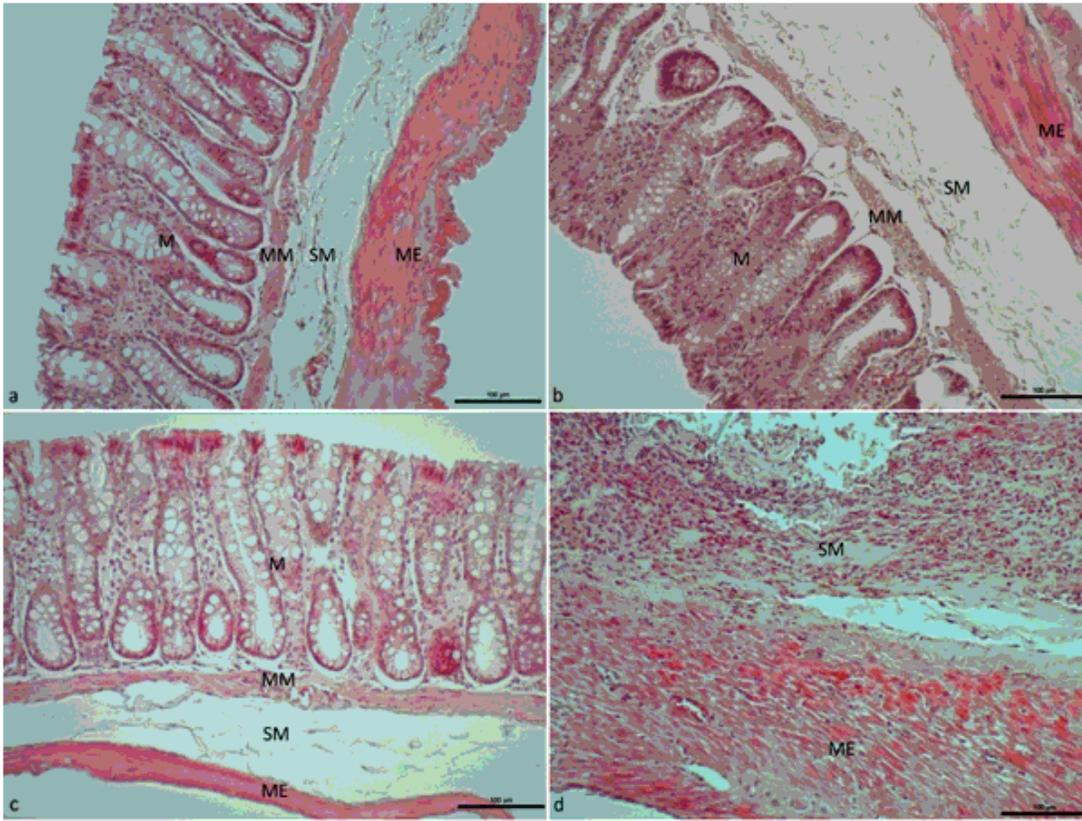


Figure 3

Boxplot for macroscopic finding according to rat groups.



a: Control Salin,
 b: VitB12 AA,
 c: Vit B12 salin,
 d: AA

M: mucosa, MM: muscularis mucosa, SM: submucosa, ME: muscularis externa (HE, Bar: 100μm).

Figure 4

Boxplot for the inflammation score average according to rat groups.

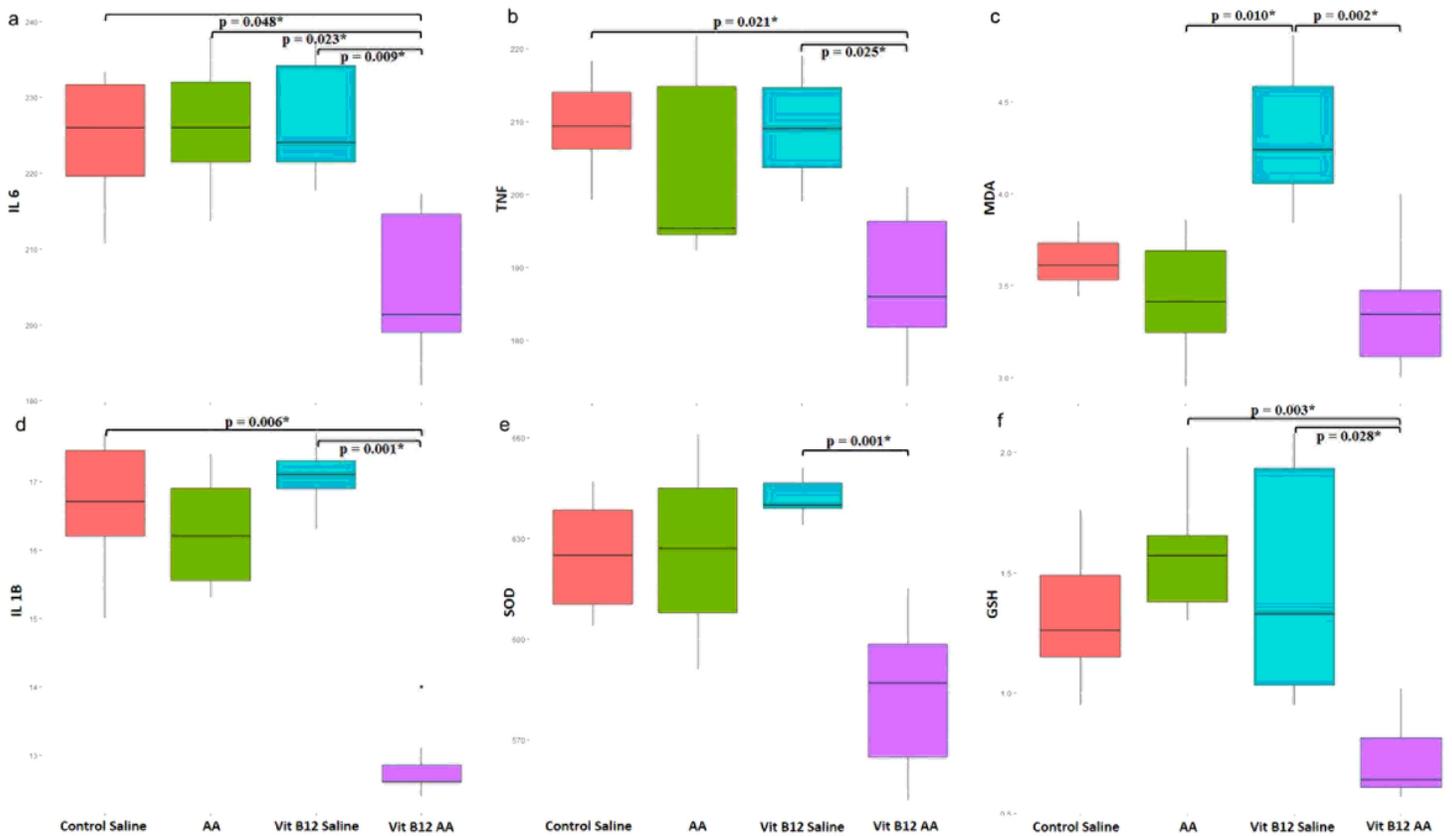


Figure 5

Boxplots for IL6 (a) and TNF α (b), MDA (c), IL1B (d), SOD (e), GSH (f) measurements according to rat groups.

Supplementary Files

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