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Anti-inflammatory and Antioxidant Effects of Thiamine in Dextran Sulfate-Induced Colitis in Rats

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Introduction

In genetically susceptible individuals, inflammatory bowel disease (IBD) is thought to occur because of an exaggerated immune response to normal intestinal flora with the contribution of multiple internal and external environmental factors [1]. Many animal models are used to understand the pathophysiology and to develop new treatment modalities in this disease [2].

The mechanisms that initiate inflammation in IBD are very variable. Toxin and infectious agents in the lumen cross the mucosal barrier and initiate inflammation in genetically susceptible individuals [2-4]. Mucosal oxidative stress caused by inflammatory cells and disruption of redox balance plays a critical role in the pathogenesis of IBD. The production of reactive oxygen metabolites is accompanied by neutrophil infiltration and activation of colitis [5].

Thiamine (Vitamin B1) is an essential vitamin for organism metabolism. It is involved in the intermediate metabolism of carbohydrates in all body tissues. In thiamin deficiency, the use of pyruvic acid and some amino acids in tissues decreases, while the use of fats increases. Therefore, it is specifically required for the final metabolism of carbohydrates and many amino acids. Thiamin is synthesized in the colon and dietary intake. Thiamine pyrophosphate, the active form of thiamine, is an essential cofactor of five enzymes involved in glucose, amino acid, and lipid metabolism [6,7].

Thiamin enables pro-apoptotic proteins of the Bcl-2 family, caspase-3 activation, and inhibition of poly- (ADP-ribose) polymerase (PARP) cleavage. It activates mitochondrial membrane potential, cytochrome c release, apoptosis-inducing factor, and then p38-MAPK, stress-activated kinases (SAPK / JNK), protein kinase C, and cytoplasmic phospholipase A2. Thiamin acting on macrophages has a potential anti-inflammatory and antioxidant effect by suppressing oxidative stress-induced NF-kappaB activation [8].

It has been reported that mild thiamine deficiency in individuals with IBD leads to many symptoms, especially fatigue [9]. As there will be problems with thiamin absorption in IBD, thiamin synthesis from the colon may also be affected [10]. The reflection of this effect, which is thought to be caused by thiamine deficiency on IBD, on tissue level can manifest as an oxidant and inflammatory stress. Thiamin supplementation may reduce this tissue damage. This study investigated the effectiveness of thiamin supplementation on inflammation and oxidative stress in the experimental IBD model.

Material And Methods

Animals

6 - 8 week-old 30 male Wistar albino rats were used in this study. All animals were housed in Dokuz Eylul University School of Medicine, experimental animal laboratories for 12 h of light, 12 h of darkness, 20–22 0C room temperature, and 40%-60% relative humidity under macro-environmental conditions and in standard rat cages until the end of the experiment. The animals were fed “ad libitum ” with drinking water and standard pellet feed. Daily body weight measurements at the same time and the amount of water they drank with water/Dextran sülfat sodium (DSS) were recorded. 3% DSS (NeoFroxx, 1138GR250, Germany) solution used for the formation of colitis in rats was prepared with fresh autoclaved sterile water every day. The experiment was conducted regarding animal rights set by the “National Institute of Health (NIH)/US A " [11]. The experiment protocol was approved by the animal care committee of Dokuz Eylul University School of Medicine (permission number HADYEK 53/20.4.2016).

Induction of colitis

The experimental protocol in the form of 3% DSS for seven days to create colitis [12-14]. A starting study was conducted with two Wistar albino male rats before starting the experiment. Because of the preliminary study, it was observed that the colitis model was formed using 3% DSS for seven days and the experiment was conducted. The presence of latent blood in the stool was investigated using the ‘Hemoccult occult blood test’ kit (Hema - Screen Occult Blood Test, Stanbio Laboratory, USA). The colitis formation was evaluated by calculating the Disease Activity Index (HAI). After sacrifice histopathological, immunohistochemical examination and ELISA testing was performed in colon tissue [15].

Thiamin hydrochloride supplementation

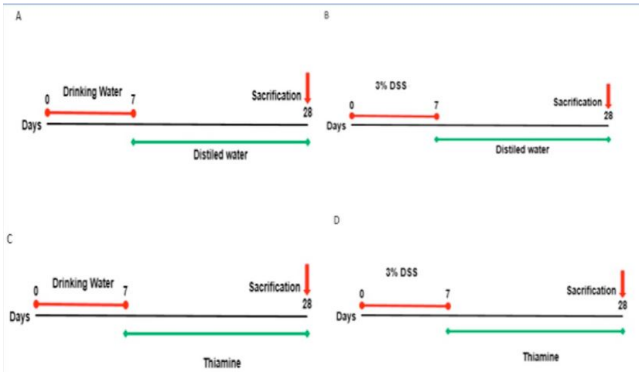
Thiamine hydrochloride (TH) via intraperitoneal or oral administration in different doses for toxic hepatitis, Wernicke Korsakoff syndrome, gastrotoxicity models was previously used in experimental rat models [16-21]. However , there were no

(16–21).

data on the use of TH in rats for experimental IBD models in the literature. We decided to use oral 600mg/kg twice daily thiamin hydrochloride for antioxidant and anti-inflammatory effectiveness.

Experiment Protocol

The rats were randomly divided into four groups, with each group consisting of 7 rats. The control group was given 1 ml of sterile distilled water with drinking water and oral gavage. 3% DSS was added to drinking water in the DSS group for the first 7 days and then 1 ml of sterile distilled water was given by oral gavage from the 8th-28th day. The thiamine group was given 600 mg/kg/dose twice a day TH oral gavage with drinking water. In the DSS + thiamine group, 3% DSS to drinking water for the first 7 days and then on the 8. day TH at a dose of 600 mg/kg/dose was added with two doses per day until sacrifice with oral gavage began (Figure 1).



The practice was done at the same time every day. The rats were placed in cages and fed as much as they wanted with rat bait.

At the end of the study, all rats were sacrificed, and their colon and spleen tissues were removed. The colon tissue samples were weighed after cleaning with a physiological saline solution and homogenized with phosphate buffer saline (pH 7.4) using an ultrasonic homogenizer (Bandelin Sonopuls, Germany). Homogenates were centrifuged at 10000 rpm +4 C for 20 minutes. The supernatants obtained as a result of centrifugation were stored at -80 C until measurements were made.

Clinical evaluation of colitis was done by calculating the Disease Activity Index (DAI), a method developed by Okayasu et al. rats DAI's was calculated and recorded daily [22]. Twenty-eighth day of the experiment, midline incisions of rats under ether anesthesia was made and the colon tissue were completely removed (from the cecum to the anus). Fecal content was cleaned and recorded by measuring the length and weight of all colonic tissue. When an increase in spleen weight as an indicator of systemic inflammation was associated with the severity of the disease, the spleen was removed and its weight was measured in aseptic conditions on the day of evaluation of animals. Macroscopic scoring was performed by opening the colon longitudinally, and changes in length were recorded due to an indicator of inflammation [23]. Histopathological damage scores were performed. In the histological examination, the parameters evaluated in colon structure were scored separately as epithelial cell loss, inflammatory cell infiltration, goblet cell damage, crypt damage, and the prevalence of damage [15,24].

Positive staining immunoreactivity in colon tissue with TNF-α and IL-6 staining was evaluated semiquantitatively as no staining: 0 (-), weak staining: 1 (+), medium staining: 2 (++), severe staining: 3 (+ + +) . Immunohistochemical staining was done by the avidinbiotin-peroxidase method with TNF-α and IL-6 staining [25-28].

The amount of MDA in the tissue was measured by spectrometric method with The Bioxytech MDA-586 (Oxis International, USA) commercial kit. The working method of the kit is based on the reaction of a chromogenic reagent with MDA at 45C. MDA values were determined for each rat from the standard curve by measuring the absorbance of 586 mm. The results obtained are expressed as µM. Glutathione values were measured with the Bioxytech GSH-420 (Oxis International, USA) kit. The working method of the kit is based on the formation of 'chromophoric thione'. Glutathione concentrations were determined for each rat by measuring absorbance at 420 nm. The results are expressed in µM.

Tissue TNF-α and IL-6 levels were also measured using the ELISA kits (BioTek ELx800, US). Tissue TNF-α values were determined according to the kit procedure with Rat TNF-α ELISA kit (Invitrogen, KRC3011, USA). IL-6 values rat IL-6 ELISA kit (Novex, Life tech. KRC0061, USA) results determined according to the procedure with the kit are expressed in pg/mL [28].

Statistical Analysis

Statistical analysis was performed using the SPSS 21.0 (SPSS Inc. Chicago, IL, USA) program. The homogeneous distribution of the data was evaluated using the Shapiro-Wilk test. According to the homogeneity of the data, the Kruskal-Wallis test or one-way variance analysis test (ANOVA) was applied for the comparison of more than two groups. The comparison of the two independent groups was done using the Student t-test was for data with a homogeneous distribution, and the Mann–Whitney U test for data with the non-homogeneous distribution. Chi-square test was used to compare group ratios. P-value <0.05 was considered statistically significant.

Results

Change in Body Weight in Rats

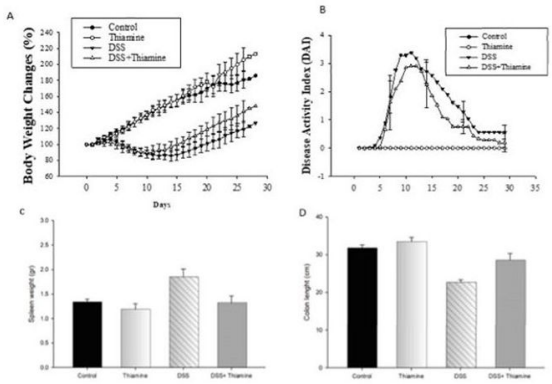
Body weight, DAI, spleen weight, colon length was determined in rats. The administration of 3% DSS for seven days induced acute colitis was observed by weight loss starting on day in DSS and DSS+the thiamin group compared with the control and thiamin group ($p<0.001$). After the 14th day, weight gain was observed in the DSS+thiamine group ($p=0.030$). On the day of sacrifice 28th day of the experiment, the weight mean gain of the DSS+thiamine group was also found to be higher than in the DSS group ($p=0.008$).

Thiamide hydrocloride (600mg/kg) suppressed DSS-induced body weight loss was obtained starting on day ($p=0.030$) and continued up to the last day of the experiment ($p=0.008$).

Seventh of day experiment weight gain in the DSS+thiamine group was less than in the control group and the thiamin group until the last day of the experiment (p<0.001). On the last day of the experiment, it was observed that the body weight gain in the thiamine group was great more than in the control group (p=0.002)(Figure 2).

Disease Activity Index, Diarrhea, Rectal Bleeding and Edema Occurrence

The score of DAI significantly increased after DSS intake, however TH treatment markedly attenuated the increased score (p<0.001). It was observed that the stool consistency of the rats in the DSS and DSS+thiamine groups softened, but there was no change in the control and thiamine groups. One of the clinical findings of colitis, bloody stool (presence of occult blood and/or macroscopic) was detected in the DSS-induced colitis groups, but not in the control and thiamine groups. Rectal edema developed in all rats in the DSS and DSS+thiamine groups. It was observed that rectal edema persisted longer in the DSS group than in the DSS+thiamine group (p=0.042) (Figure 2).



Spleen Weights, Colon Length and Weights

The shortening of the colon and swelling of the spleen is identified the severity of colorectal inflammation [32].

The mean spleen weight of the rats in the DSS group was higher than in the control group (p=0.045), and TH treatment significantly decreased the mean spleen weight of the thiamine-treated group (p=0.024), and in DSS+thiamine group (p=0.035) (Figure 2).

Significant shortening of the colon length was observed in the DSS group rats (Figure 2).

The length of the colon tissue decreased in the DSS group compared to the control group (p<0.001), the thiamin group (p<0.001), and the DSS+ thiamine group (p=0.008). There was no difference in the column length between the DSS+thiamine and control groups (p=0.345).

Mean colon weight was decreased in the DSS group compared to the control group (p<0.001), thiamin group (p<0.001), the DSS +thiamine group (p<0.001). Differences in column weights were not observed between the control and thiamine groups with the DSS+Tthiamin group (p>0.05) (Figure 2).

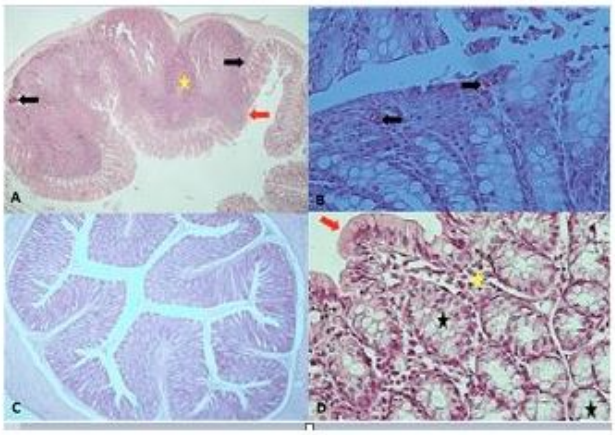
Histopathological Analysis

Histological Damage Scoring

The severity of colonic inflammation and ulceration was evaluated by histological analysis using H&E staining. It was found that the histological score in the DSS+thiamine group was less than in the DSS group (p<0.001). There was no difference between the control and the thiamin group (p=0.142).

It was observed that, the surface epithelium showed continuity in many areas in the DSS+thiamine group, while the crypt and surface epithelium goblet cell structures were similar to the control group. Also, mild edema has was detected in the inter-crypt lamina propria. Inflammatory cell infiltration was significantly reduced and mononuclear cell communities and congestion was not observed.

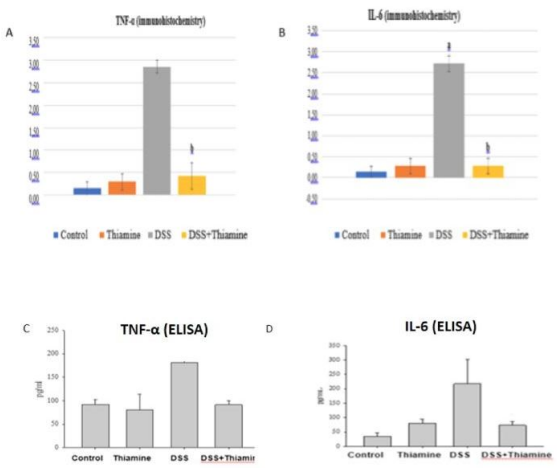
In periodic acid-Schiff (PAS) staining of the colon, severe goblet cell loss, and a significant reduction in the number was observed in the mucosal surface epithelium and crypts in the DSS group. In the thiamine group, the density of goblet cells in the surface epithelium and crypts was similar to the control group. In the DSS + thiamine group, it was observed that there was an increase in goblet cells in the surface epithelium and crypts and a close appearance in the control group (Figure 3).



TNF-α and IL-6 levels in the colon tissue by ELISA method

TNF-α values of the DSS group were found to be higher compared to the control, thiamin, and DSS+thiamine groups (p<0.05).There were no differences between the control and the thiamine groups (p>0.05).

IL-6 levels were found to be low in the DSS+thiamine and thiamine groups compared to the DSS group (respectively, p=0.032, p=0.041). There were no differences between the control and the thiamine groups (p>0.05) (Figure 4).



Evaluation of oxidative stress and inflammation

Malondialdehyde (MDA) and Glutathione (GSH) levels in colon tissue

Malondialdehyde levels in colon tissue were low in control, thiamin, and DSS+thiamine groups compared to the DSS group ($p<0.001$).Tissue GSH levels were found to be low in the DSS group compared to the control, thiamin, and DSS+thiamine groups ($p<0.05$).

Discussion

A few studies on the literature have shown that thiamin has antioxidant and anti-inflammatory effects. Preclinical and clinical findings draw attention to the possibility of repositioning B vitamins, drugs with a good safety profile, to treat patients with chronic painful and inflammatory diseases [33]. High-dose oral thiamine administration has been shown to inhibit the hexosamine biosynthesis in rats and prevent the development of cardiomyopathy [34]. Another study by Babaei et al. has shown to prevent diabetic nephropathy [35]. In animal models in which hepatotoxicity is induced by methotrexate, it has been shown that thiamine has antioxidant and anti-inflammatory effects, both by the level of GSH in the tissue and the amount of MDA in the tissue. Furthermore, thiamin has been demonstrated to have antioxidant and anti-inflammatory effects in different animal models where hepatotoxicity is generated [36].

The DSS-induced colitis model occurs more slowly in young rats compared to older rats. This occurs because young rats consume relatively less feed and water. The most effective age range was determined to be 6–8 weeks for colitis model. DSS forms colitis in both female and male rats. It occurs more severely and faster in males [37]. For these reasons, we preferred 6–8 weeks old male Wistar albino rats of of the male in our study.

In humans, Magee et al. demonstrated that there was no clinical difference in the severity of the disease between patients with IBD who received B-complex vitamin supplements and controls [38]. Also, they demonstrated that some food habits such as caffeine consumption, high carbohydrate food intake, which causes thiamin deficiency, increase the severity of the disease [38]. In our study, anti-inflammatory and antioxidant effects of oral TH supplementation were shown in an experimental colitis model.

A decrease in DAI score was observed in the DSS and DSS +thiamine groups within days after the termination of DSS administration. A decrease in DAI score in the DSS+ thiamine group was observed earlier and more significantly than in the DSS group. This result suggests the improvement effects of TH on clinical manifestations of colitis.

The spleen weight in the DSS + thiamine group was less than in the DSS group, and the colon length was greater. We found that TH provides close histological improvement compared to the control group.

Excessive release of TNF-α stimulates the acquired immune system, leading to increased colon damage by infiltrating large numbers of neutrophils and macrophages into the colon mucosa [39]. For this reason, many immunosuppressive agents are used to suppress TNF-α production for treating IBD. IL-6 also plays an important role in the pathogenesis of IBD, being responsible for the formation of inflammation and the release of many other proinflammatory cytokines [40]. High levels of TNF-α and IL-6 in the colon tissue were also shown in the DSS-induced colitis model in rats [41]. In our study, it was observed that rats in the DSS group had high levels of TNF-α and IL-6 in the colon tissue compared to the control group, thiamin group, and DSS +thiamine group. It suppresses TNF-α and IL-6 release in tissue by TH supplementation.

The use of antioxidants is common in healthcare, such as treating cardiac, neurological, gastrointestinal diseases [42,43]. Oxidative stress is one of the critical factors involved in the pathogenesis of IBD. Oxidative stress damages cellular macromolecules such as DNA, lipids, and proteins, causing organ and tissue damage [43]. Malondialdehyde, the end produc t in lipid peroxidation, plays an important role in the pathogenesis of IBD by overcoming the defense mechanisms in the tissue, leading to damage [44]. Studies show a significant increase in MDA levels in the colon tissue in colitis models created in rats with DSS [43-44]. In our study, it was observed that there was a significant increase in MDA levels in DSS groups compared to thiamin and control groups. Also, lower levels of MDA in the DSS +thiamine group than in the DSS group were detected. Similar results were also found in DSS induced colitis models with different antioxidants [23]. In our study , we showed that TH supplementation suppresses oxidative stress.

Glutathione peroxidase and GSH, antioxidant enzymes, play an important role in lipid peroxidation [45]. Haiyan and colleagues found lower activity of these enzymes in the DSS induced colitis model and it was thought that there was a relationship between this effect and the severity of colitis [23]. Similar to previous studies, lower activity of GPX and GSH in colon tissue was found after DSS administration [46]. In our study, a significant increase in GPx and GSH activity after TH supplementation suggests that TH stimulates the antioxidant enzyme activity and reduces the severity of colitis.

In conclusion, the results presented here indicate that there are antioxidant and anti-inflamatory effects of TH on

experimental colitis. The data in this study relate only to DSS induced rat colitis model, we believe that they will be useful to support the set-up of a randomized controlled trial evaluating the therapeutic efficacy of thiamin supplementation in IBD.

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