Hematoprotective Effect of N(G)-Nitro-L-Arginine Methyl Ester and Lycopene on Hematoxicity in Trinitrobenzene Sulfonic Acid–Induced Colitis in Rat

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Abstract

Anemia is a neglected manifestation of inflammatory bowel disease (IBD) although it is commonly observed in IBD patients. Attempts to overcome anemia in IBD would help not only general well being of the patients but also minimize disease consequences. In this experimental study, the possible hematoprotective effects of lycopene and NG–nitro-L-arginine methyl ester (L-NAME) on colitis induced by TNBS were analyzed. 112 rats were assigned to 16 groups; control group, intrarectal 120 mg/kg TNBS group, intraperitoneal 40 mg/kg L-NAME group, 1 mg/kg olive oil group, 5 and 10 mg/kg lycopene groups. Each experimental group was divided into 3 subgroups according to duration of treatment. On the very first day of treatment number of erythrocytes decreased in all groups except TNBS treated group whereas leukocyte numbers increased in all groups except TNBS treated group pointing out an inflammation. The number of platelets decreased in all study groups with the exception of TNBS group. On the second day, while erythrocyte and platelet numbers increased in all but not in TNBS group, leukocytes decreased in all the groups. On the third day, erythrocyte and platelet numbers increased in all groups except for the 10 mg/kg lycopene group. While the number of leukocytes decreased in the 10 mg/kg lycopene group, it remained the same in the other groups as those observed on the second day. These results show that lycopene could have effects on hemopoiesis as well as in prevention of anemia in IBD.

Keywords: TNBS, colitis, lycopene, hematoprotectivity

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Introduction

In addition to intestinal symptoms inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, there are number of extra intestinal manifestations [1,2]. One of the hallmarks of active episodes of colitis is the infiltration of large numbers of phagocytic leukocytes into the mucosal interstitium. Enhanced inflammatory infiltrate is extensive in mucosal injury, giving rise to production and release of large quantities of reactive oxygen metabolites (ROM) such as superoxide and hydrogen peroxide. Studies on colitis have revealed that free radicals (peroxynitrite and oxygen radicals) stemming from nitric oxide (NO) exacerbate the existing damage [3]. NO is synthesized from L-arginine occurring in mitochondrial inner membrane by means of nitric oxide synthase (NOS). The NOS enzyme belongs in the family of cytochrome p-450 protein, and is suppressed by some arginine analogues like L-NAME [4]. An experimental study by Masubuchi et al (2008) determined an increase in concentrations of NO and its metabolites in the portal area in colitis. In addition to classic ROM, activated neutrophils and monocytes also secrete hemoprotein myeloperoxidase into extracellular medium where it catalyzes oxidation of chloride ions via hydrogen peroxide to yield a highly reactive oxidizing and chlorinating agent named as hypochlorous acid. This acid has been shown to degrade gastrointestinal mucin, enhance mucosal permeability, and injure intestinal epithelial cells [7]. Furthermore, systemic manifestation of IBD may also include malnutrition and anemia [1] and anemia is a common extraintestinal complication of IBDs.

Inflammation has negative effects on three major steps of normal erythropoiesis and can therefore also lead to anemia. These effects are: (i) an immunity-driven diversion of iron traffic leading to retention of the metal in macrophages and thus to iron-deficient erythropoiesis; (ii) blunting of the biological activity of erythropoietin, the major erythropoiesis-stimulating hormone; and (iii) inhibition of the differentiation and proliferation of erythroid progenitor cells [8]. During inflammatory processes, however, the biological half-life of erythrocytes is reduced as a consequence of oxidative stress and lipidperoxidation, thus promoting erythrophagocytosis and reducing iron recirculation [9, 10]. In addition to this, pro-and anti-inflammatory cytokines contribute to iron retention within monocytes/macrophages even more by stimulating iron uptake pathways while inhibiting ferroportin transcription in these cells [11]. All these events lead to retention of iron within

phagocytes and to the development of functional iron deficiency, meaning that although iron is abundant in the body the metal is not available for erythropoiesis [8]. In IBD, this scenario is typically associated with iron deficiency which is a consequence of recurrent bleeding episodes from ulcerated intestinal mucosa. The combination of functional iron deficiency due to anemia of chronic disease (ACD) and chronic blood loss is the most common finding in IBD. In fact, whenever there is intestinal inflammation, subsequent blood loss (through the ulcerated mucosa) is to be expected. Therefore, IBD-associated anemia is the prototype of iron deficiency combined with ACD. In addition to the limited availability of iron for erythropoiesis, the cytokine mediated down-regulation of erythropoietin-receptor expression on progenitor cells, impaired biological activity of erythropoietin, reduced expression of other hematopoietic growth factors such as stem cell factor, as well as toxic effects of radicals such as nitric oxide or superoxide anion, further inhibit erythroid progenitor cell proliferation [9,12]. Hence, giving anti-inflammatory drugs to patients with IBD and curing the disease with antioxidant substances, such as lycopene, seems to be as important as iron and vitamin replacement.

Recent studies have demonstrated that Lycopene exhibits health beneficial effects by virtue of its antioxidant activity [5]. Lycopene is one of the most potent antioxidants, with a singlet-oxygen-quenching ability twice as high as that of β-carotene and 10 times higher than that of α-tocopherol [6]. On the other hand, lycopene has been reported to prevent reactions which result in inflammation by suppressing synthesis of such proinflammatory molecules as prostaglandin, prostacycline, thromboxane, and leukotriene thanks to regulation of cyclooxygenase and lipooxygenase [13]. Some studies into lycopene determined that it decreases levels of malondialdehyde (MDA), a product known to correlate with oxidative damage thanks to its association with the oxidation of fatty acid, and that lycopene increases the activities of such antioxidants as superoxide dismutases (SOD) and glutathione peroxidase (GP) [14]. A studyclearly demonstrate that lycopene is able to suppress NDEA-induced oxidative stress in rats [19].Altough a lot of studies were focused on the antioxidant and anti-inflammatory properties of lycopene its effects on diseases such as IBD or on extraintestinal manifestations of IBD (anemia) have not been identified. The present study aims to determine protective effects of lycopene on peripheral blood cells in Trinitrobenzene Sulfonic Acid (TNBS) induced colitis.

Materials and Methods

This 112 male Sprague Dawley rats weighing between 220 and 250 gr were used in this study provided by TICAM, Medical and Surgical Experimental Research Centre at Eskisehir Osmangazi University. Ethics committee approval was obtained before this experimental study could be done (PR-07-03-15-2, Eskisehir Osmangazi University). Rats were divided into 16 groups, a single group had 7 rats. Experimental groups were named as TNBS (120 mg/kg), L-NAME (40 mg/kg), olive oil (1 mg/kg), Lycopene (5 mg/kg), lycopene (10 mg/kg) and control group. TNBS and L-NAME doses were determined based on previous study[17]. Experimental study was designed in a way that each group of animals were divided into 3 subgroups according to the duration of treatment such as day 1, day 2 and day 3 respectively.

TNBS, L- NAME and lycopene were provided by Sigma. A dose of TNBS (120 mg/kg) was prepared for the study groups using methanol of 50%. Acute experimental colitis was induced in rats with a plastic cannula that was inserted into the anus of the rats for nearly 8 cm, through which TNBS could be administrated appropriately [15].

All groups received TNBS and the day after TNBS had been administrated, different study groups were arranged in a way that one group received 40 mg/kg of L-NAME, the other one 5 mg/kg other group having 10 mg/kg of lycopene and last group had olive oil applied intraperitoneally (i.p.) for three days in a row. Once TNBS had been administrated, the rats in L-NAME, olive oil and lycopene groups were anesthetized with ether before being dissected on the first, second and third days of the application. The blood samples were taken for analyses.

Analysis of blood samples

The blood specimens were withdrawn during Ketamine and Xylazine anesthesia. Cardiac blood was withdrawn into tubes containing 3.8% of sodium citrate by the intracardiac method on the first, second and third days, and then it was detected immediately in a blood count machine (Hamewat 850).

Statistical evaluation

The results were expressed as means ± Standard error of the mean. Statistical analysis was performedby using one-way analysis of variance (ANOVA), followed by Tukey's multiple range test, and $P < 0.05$ was accepted as a statistical significance. Triplicate samples from each animal were taken, and the results were expressed as a mean for each animal.

Results

The bloods samples obtained from the heart were processed in a blood count device commercially named as Hamewat, which is specially designed for animals. Relevant tables and charts were arranged in accordance with the statistical analyses of peripheric blood samples (erythrocytes, leucocytes, and thrombocytes) using the SPSS 9.0 package software.

Comparison of erythrocytes values

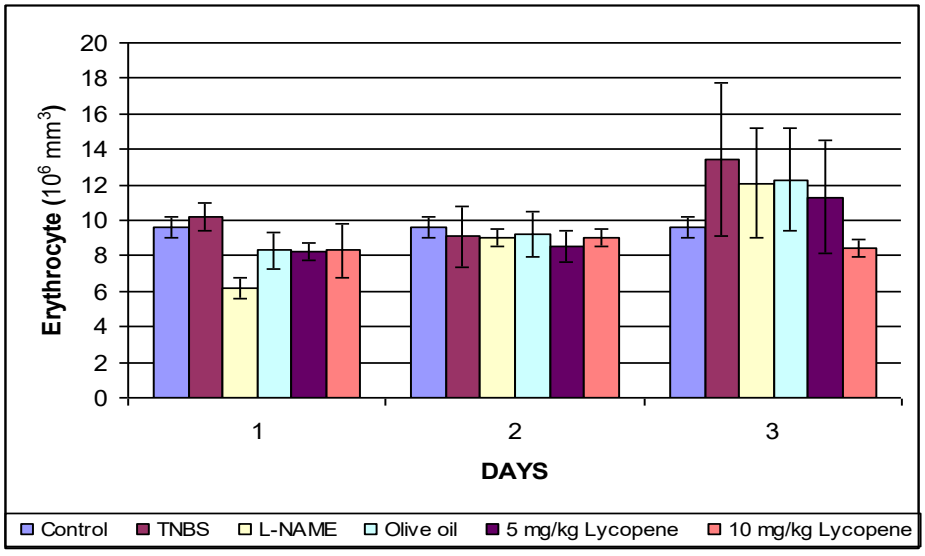

Figure 1. Erythrocyte levels observed in the control and study groups on the first, second and third days.

Erythrocyte levels on the 1st day

It was observed that all the groups apart from the ones given TNBS exhibited a statistically significant decrease in the number of erythrocytes when compared to the control group ($p<0.001$). On the other hand, the TNBS only groupsshowed a statistically significant rise in the number of erythrocytes when compared to the control and the other study groups

($p<0.001$). As to the L-NAME group, the number of erythrocytes showed a statistically significant decrease in comparison with the olive oil group (1 mg/kg) and the lycopene groups (5 and 10 mg/kg) ($p<0.001$). The olive oil group showed no significant difference from the Lycopene groups of 5 or of 10 mg/kg ($p>0.05$).

Erythrocyte levels on the 2nd day

While there was a little variation in the erythrocyte levels between the control, TNBS, L-NAME, olive oil and Lycopene groups, this variation achieved no statistical significance ($p>0.05$).

Erythrocyte levels on the 3rd day

While there was rise of statistical significance in the number of erythrocytes in the groups of TNBS, L-NAME, olive oil and the Lycopene group given 5 mg/kg of lycopene in comparison with the control group ($p<0.05$), 10 mg/kg of lycopene group showed a decrease in the number of erythrocytes compared to the control group; however, this decrease was of no statistical significance ($p>0.05$). Number of erythrocytes was found to be higher than all the other groups, though it was deemed statistically insignificant ($p>0.05$). No statistically significant difference could be found in the number of erythrocytes between the groups of L-NAME, olive oil and the lycopene group given 5 mg/kg of lycopene ($p>0.05$); however, there was a statistically significant decrease in number of erythrocytes in comparison with the lycopene group given 10 mg/kg of lycopene ($p<0.05$). As to the olive oil group, we could find no statistically significant difference between the lycopene group given 5 mg/kg of lycopene and olive oil group ($p>0.05$), but there was a significant rise in the number of erythrocytes in the lycopene group given 10 mg/kg of lycopene ($p<0.05$). As for the lycopene groups, 5 mg/kg of lycopene had a rise in the number of erythrocytes compared to the one given 10 mg/kg of lycopene, which was deemed to be statistically different ($p<0.05$).

Comparison of the levels of leucocytes

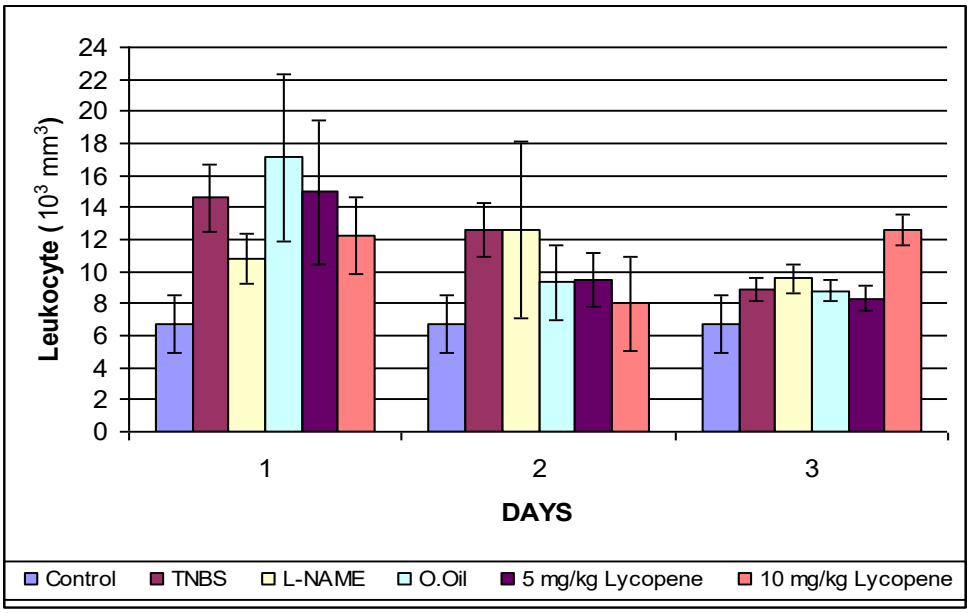

Figure 2. Levels of leukocytes of the control and study groups on the 1st, 2nd and 3rd days.

Leukocyte levels on the 1st day

The number of leukocytes in TNBS, L-NAME, olive oil, 5 and 10 mg/kg lycopene groups were increased compared to the control group and this rise was found to be statistically significant ($p<0.001$). Though there was a rise and fall in the number of leukocytes in the TNBS, L-NAME, olive oil, 5 and 10 mg/kg Lycopene groups, these differences were statistically insignificant ($p>0.05$).

Leukocyte levels on the 2nd day

When compared to the control group, there was a high rise in the number of leukocytes in the L-NAME and TNBS groups, which was statistically significant ($p<0.001$), the rise in leukocyte numbers in olive oil and lycopene groups reached no statistical significance ($p>0.05$). On the other hand, TNBS, L-name, olive oil and lycopene groups showed no increase in the number of leukocytes.

Leukocyte levels on the 3rd day

The rise in leukocyte numbers was much lower in the TNBS group compared to the Lycopene group given 5 mg/kg of lycopene and the olive oil group, which was determined to be

statistically significant ($p<0.05$). On the other hand, the number of leukocytes in 10 mg/kg of lycopene was very high compared to the TNBS group ($p<0.001$). No statistically significant difference was found in the number of leukocytes as far as the olive oil, L-NAME, and 5 mg/kg lycopene group are concerned ($p>0.05$). No statistically significant difference could be found between the groups of olive oil, L-NAME, and 5 mg/kg of lycopene group in terms of the number of leucocytes. However, there was a significant increase in the number of leukocytes in 10 mg/kg of lycopene group in comparison with 5 mg/kg of lycopene group, L-NAME, and the olive oil group ($p<0.001$).

Comparison of the values of thrombocytes

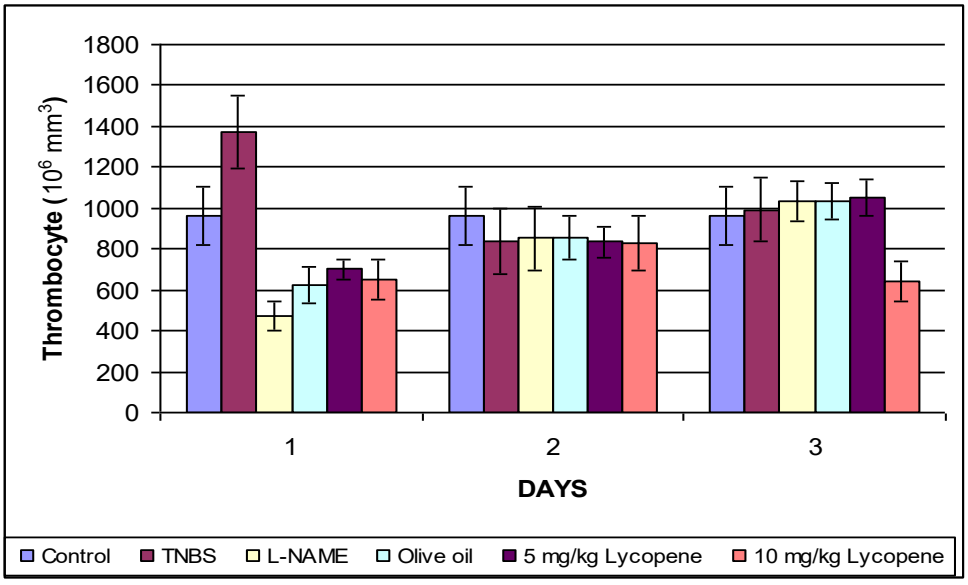

Figure 3. Thrombotic values observed in the control and the study groups on the 1st, 2nd and 3rd days.

Thrombotic values on 1st day

When compared to the control group, the TNBS group showed a significant rise in the thrombocyte values ($p<0.001$). On the other hand, the L-NAME, olive oil and lycopene groups were found to have a significant fall in the thrombocyte values ($p<0.001$). In the mean time, there was a decrease in the number of thrombocytes in the L-NAME group in comparison with the others ($p<0.001$). However, we could find no significant difference between the olive oil and lycopene groups in terms of the thrombocyte values ($p>0.05$).

Thrombocyte values on 2nd day

No statistically significant difference could be found between the number of thrombocytes observed in the control and the study groups ($p>0.05$).

Thrombocyte values on 3rd day

There was a statistically significant difference between only the control and 10 mg/kg of lycopene group ($p<0.001$). In other words, no statistically significant difference could be found between the thrombocyte numbers of the remaining groups ($p>0.05$).

Discussion

This study determines the cytoprotective effect of L-NAME and lycopene on TNBS-induced inflammatory status in a rat model of colitis. Results show that erythrocytes, leukocytes and thrombocytes increased significantly in the rats given TNBS on the first day. When the TNBS group was compared to the control group for the peripheric blood parameters, it was determined that the number of erythrocytes and thrombocytes in the TNBS group rose significantly on the 1st and 3rd days but there was a slight fall in this number on the 2nd day. As to the number of leukocytes, it was determined to be higher on the first two days, which seemed to indicate that the severity of inflammation was higher than that of the control group throughout our experimental study. The number of the erythrocytes observed in the L-NAME group on the first two days was much smaller than that of the control group but it rose to a significant extent on the 3rd day. However, the difference in the number of thrombocytes throughout the present study was of no significance. On the other hand, the number of leukocytes was observed to have increased throughout the study, though this increase was low on the 3rd day.

The number of leukocytes in the olive oil group remained high throughout the study, although the increase observed was higher on the 1st day. The number of erythrocytes that decreased on the 1st day of the experiment grew increasingly bigger on the 2nd and 3rd days, and exhibited a parallel pattern in reaction to the response of leukocytes to inflammation. As to prevent anemia or to exhibit antioxidant effects, it was determined that the lycopene groups were more capable of maintaining the number of erythrocytes on the first two days than was the L-NAME group. The fact that the number of erythrocytes and thrombocytes went down

on the 3rd day in 10 mg/kg of lycopene group seems to suggest that anemia lost its severity and thus the inflammation diminished to a significant extent. This being the case, 10 mg/kg lycopene could be protective against anemia, and ultimately against inflammation, than L-NAME and olive oil. The rise in the number of leukocytes on the 1st and 2nd days could be attributed to the severity of the inflammation observed throughout the study. On the other hand, the rise in the number of thrombocytes in the TNBS group on the very first day could be due to acute inflammation and bowel hemorrhage. The fact that the number of leukocytes showed an upward trend throughout the study in this group could be an indication that the immune response to inflammation was severe. The rise in the number of erythrocytes on the 3rd day in the TNBS group could be not because of stimulation of erythropoiesis by TNBS but because of the release of stored erythrocytes. That the same effect could be achieved by L-NAME and olive oil has already been reported in experimental studies [16], which shows that the blood cell count get normalized as the severity of colitis diminishes and that this effect was not due to new formation of blood cells but release of the stored ones to the bloodstream. In fact, peripheral blood results suggest that lycopene helped to reach cell numbers comparable to control levels particularly on the 2nd and 3rd days of the study. Yaping et al. (2002) reported that lycopene has both anti-inflammatory and anticoagulant effects [18]. The number of the thrombocytes in our lycopene groups remained low throughout the whole experiment, with the exception of a small rise observed in the group given a dose of 5 mg/kg of lycopene on the third day, showing a possible anticoagulant effect of lycopene.

In conclusion, findings of this experimental study show that lycopene could have a role on hematopoiesis as well as on the prevention of anemia in colitis however, in order to shed further lights on the effect of lycopene on chronic colitis, long term studies are needed.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the Journal of Crohn's and Colitis.

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