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Protective effect of tryptophan against dextran sulfate sodium- induced experimental colitis

Toru SHIZUMA, Hidezo MORI, Naoto FUKUYAMA

Department of Physiology, Tokai University, School of Medicine, Isehara, Kanagawa, Japan

Background/aims: *Little is known about the anti-colitis effect of tryptophan or its metabolites. Here, the protective effect and its mechanism of tryptophan administration on dextran sulfate sodium -induced colitis in mice was studied.* ***Materials and Methods:*** *Twenty C57black6 female mice were equally divided into the control group, and treatment group. The control group received a standard CE-2 diet, while the tryptophan group received a CE-2 diet containing 0.5% l-tryptophan. After one week on this diet, all mice were orally administered a solution of 3.5% dextran sulfate sodium for 12 days to induce colitis. Changes in body weight and bloody stool frequency were monitored during dextran sulfate sodium administration. At 12 days post initial dextran sulfate sodium administration, all mice were sacrificed and the histology of their colonic tissue was examined. The nitrotyrosine levels in colonic tissues in both groups, and nitrate and nitrite levels in the urine of the control group, the tryptophan group and the group of mice without dextran sulfate sodium administration was measured.* ***Results:*** *The tryptophan group showed significantly attenuated body weight loss, bloody stool frequency and ameliorated histological changes of colitis. While tryptophan treatment significantly reduced nitrotyrosine level in the colonic tissues, there was no significant reduction in urine nitrate and nitrite levels compared with the (dextran sulfate sodium-induced) control group.* ***Conclusion:*** *Tryptophan treatment ameliorated dextran sulfate sodium-induced colitis in this study. One of the anti-colitis mechanisms of tryptophan treatment is attributable to an anti-nitration effect, and may not be via the suppression of nitric oxide generation.*

Key words: Tryptophan, ulcerative colitis, nitrotyrosine, peroxynitrite

Dekstran sülfat sodyuma bağlı deneysel kolitte triptofanın koruyucu etkisi

Amaç: *Triptofan veya metabolitlerinin anti-kolitik etkinliği ile ilgili fazla bilgi yoktur. Burada, farelerde dekstran sülfata bağlı kolitte triptofan uygulanmasının koruyucu etkisi ve mekanizması incelenmiştir.* ***Gereç ve Yöntem:*** *Yirmi C57siyah6 dişi fare, standart CE-2 diyet ve %0.5'lik l-triptofan içeren CE-2 diyeti alan triptofan grubuna ayrıldı. Bir hafta sonra, tüm farelere %3.5'lik dekstran sülfat sodyum oral yoldan 12 gün verilerek kolit indüklendi. Dekstran sülfat sodyum verilirken vücut ağırlığı ve kanlı defekasyon sıklığı takip edildi. İlk dekstran sülfat sodyum uygulamasından 12 gün sonra tüm fareler sakrifiye edildi ve kolon dokusundaki histolojik bulgular değerlendirildi. İki gruptaki kolonik nitrotirozin düzeyleri ölçüldü. Ayrıca kontrol, triptofan ve dekstran sülfat sodyum uygulanmayan farelerde idrar nitrat ve nitrit düzeyleri ölçüldü.* ***Bulgular:*** *Triptofan grubunda vücut kilo kaybı, kanlı gayta sıklığı ve kolite bağlı histolojik değişiklikler anlamlı şekilde düzelmişti. Triptofan tedavisi kolonik dokulardaki nitrotirozin düzeylerini kontrol grubuna göre (dekstran sülfat sodyumla indüklenmiş kolitte) anlamlı şekilde azaltmış ve idrar nitrat ve nitrit seviyelerini düşürmüştü.* ***Sonuç:*** *Bu çalışmada, triptofan tedavisi dekstran sülfat sodyuma bağlı koliti düzeltmiştir. Triptofan tedavisinin anti-kolitik etki mekanizmalarından birisinin, nitrik oksit oluşumunda az.*

Anahtar kelimeler: Triptofan, ülseratif kolit, nitrotirozin, peroksinitrit

INTRODUCTION

Ulcerative colitis (UC) is the typical progression of chronic inflammatory bowel disease (IBD). The causes of UC are not fully understood, but multiple genetic factors (1), immune responses in the colon, intestinal flora, oxidative stress (2,3), are thought to influence its severity and pathogenesis. The disease is generally treated with prednisolone or immuno-suppressive agents, but the treatment

Address for correspondence: Toru SHIZUMA
Department of Physiology, School of Medicine, Tokai University,
Isehara, Kanagawa, Japan
Phone: +81 463 93 11 21
E-mail: shizuma@is.icc.u-tokai.ac.jp

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of UC can be potentially toxic and long-term fasting and total parenteral nutrition is sometimes necessary for patients. The anti-colitis effects of probiotic or symbiotic administration have been reported in patients with limited mild UC (4,5). However, effective nutritional therapies for UC have not been fully investigated in contrast to Crohn's disease. Therefore, it may be beneficial to investigate the safety effects of dietary factors in the management of UC.

Tryptophan, an essential amino acid in mammals, is a precursor for biosynthesis of serotonin via the kynurenine pathway in human beings. Recently, the protective effect of L-tryptophan administration on dextran sulfate sodium (DSS)-induced colitis by the reduction of pro-inflammatory cytokines and activation of apoptosis initiators was reported (6). However, the anti-oxidative and anti-nitration effects of tryptophan or its metabolites have not previously been investigated.

Oxidative stress or nitration stress are involved in the severity and pathogenicity of UC (3). Enhanced release of reactive oxygen species (ROS) such as superoxide, hydroxyl radical and reactive nitrogen species (RNS) such as peroxynitrite via nitric oxide (NO), play aggravated roles in both clinical UC and DSS-induced colitis animal models (7). The excess generation of superoxide or NO is often cytotoxic and can induce tissue damage. Moreover, superoxide reacts rapidly to NO and induces the generation of peroxynitrite which is a strong cytotoxic, leading to severe tissue damage (8,9). Nitrotyrosine is widely used as a marker for the generation of peroxynitrite.

The relationship between the ameliorative effects against colitis and the anti-oxidative or nitration effects of tryptophan administration have not been investigated, although one study has reported the protective effect of tryptophan administration against DSS-induced colitis in an animal model (6). In this study, the mechanism of the protective effect of tryptophan administration on DSS-induced colitis model in mice was examined using an assay of nitro compounds in light of its anti-oxidative and nitration effects.

MATERIALS AND METHODS

Experimental procedures were reviewed and approved by the Animal Experimentation Committee, School of Medicine, Tokai University, Japan.

Experimental animal model

Twenty C57black6 female mice (6 weeks of age) were obtained from CLEA Japan Inc. (Tokyo, Japan) and bred under specific pathogen-free conditions at a room temperature of 25 °C, with a 12-h light/dark cycle.

These mice were randomized into two dietary groups. The control group (10 mice) received a standard CE-2 diet and the tryptophan group (10 mice) received a CE-2 diet containing 0.5% l-tryptophan. The standard CE-2 diet was supplied by CLEA Japan Inc. (Tokyo, Japan), while the CE-2 diet containing l-tryptophan was supplied by Sakamoto Kurozu Inc. (Kagoshima, Japan). The diets were started a week prior to the initial administration of DSS. In both groups, a 3.5% solution of DSS (Sigma-Aldrich, St. Louis, USA) in water was orally administered for 12 days to induce colitis. In addition to the 2 groups, 10 mice receiving a standard CE-2 diet did not receive DSS.

Evaluation of manifestations in mice

Changes in body weight and bloody stool frequency were monitored as indices of severity of colitis every 2 days for 12 days after the initial administration of DSS. The mean body weight and standard deviation (SD) was calculated. Body weight changes after DSS administration were compared as a calculated percentage of the basal body weight before DSS administration (taken as 100%). Bloody stool frequency in mice after DSS administration is shown as a percentage in each group (number of animals with bloody stool/ total number of animalsx100%). All mice were sacrificed under isoflurane anesthesia (Wako Pure Chemicals, Osaka, Japan) at 12 days after initial DSS administration. The proximal middle colon was then resected.

Histological examination

For microscopic examination, resected colonic tissues from all animals were fixed in buffered formaldehyde, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H.E.).

Nitrotyrosine assay in colonic tissues

Nitrotyrosine levels of resected colon tissues at 12 days after initial DSS administration were measured using a commercial enzyme-linked immunosorbent assay (ELISA). Values are presented as a mean with the SD. Resected colon tissues were homogenized by centrifugation at 20,000×g (20 min), and the supernatant was examined with a Nitrotyrosine ELISA Kit, NWLSS (2×96 well) (Funakos-

hi Co., Osaka, Japan) according to the manufacturer's instructions. The absorbance at 450 nm was measured.

Nitrate and nitrite assay in urine

Urinary excretion/day (measured for 11-12 days after initial DSS administration) of nitrate and nitrite (NOx) as a parameter of the bioavailability of NO was measured by the Griess method (Griess reagent kit; Invitrogen Japan K.K.,

Tokyo, Japan) for all three groups; the (DSS-induced) control group, the tryptophan group, and the group of mice which received the standard CE-2 diet without administration of DSS (each group; n=10).

Statistical analysis

The significance of differences in body weight at 2-12 days after administration of DSS and nitrotyrosine levels between the two groups was evaluated by means of unpaired t-tests. Frequency of bloody stool at 2 -12 days after DSS administration was analyzed using contingency tables.

Differences in NOx levels among the three groups were statistically analyzed by means of one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc test. The criterion of significance was set at $p<0.05$.

RESULTS

Body weight reduction was significantly attenuated in the tryptophan group throughout days 2-12 after the initial DSS administration, compared with the control group ($p<0.01$) (Table 1).

Bloody stool was observed in 9 out of 10 mice (90%) in the control group on day 2, and in all them (100%) during days 4-12. Tryptophan treatment significantly ($p<0.01$) reduced the frequency of bloody stool throughout days 2-12 compared with the control group, and the bloody stool frequency at day 12 was only 10% (Table 2).

HE staining of resected colon tissue revealed epithelial abrasions, cryptal disturbance, and inflammatory cell infiltration in mucosa and submucosal areas of colon in the control group (Figure 1A). Again, tryptophan treatment remarkably suppressed these changes (Figure 1B).

Nitrotyrosine levels in resected colonic tissues was significantly ($p<0.01$) reduced to 61.1±11.0 ng/g protein in the tryptophan group, Compared to 87.8±7.4 ng/g protein in the control group (Figure 2).

NOx levels in urine (μM/day) in the (DSS-induced) control group (821±181) and the tryptophan group (780±162) were significantly increased when compared to mice without DSS administration group (521±82).

However, there were no siginificant differences between the (DSS-induced) control group and the tryptophan group (Figure 3).

DISCUSSION

Our study revealed that tryptophan treatment ameliorated DSS-induced colitis in mice, and significantly reduced nitrotyrosine level in colon tissues, did not influence the level of NOx in urine.

Nitrotyrosine is produced in vivo via two pathways, i.e., from the reaction of tyrosine with peroxynitrite, which is generated from superoxide and NO (8), and from the reaction of tyrosine with

Table 1. Changes of body weight after administration of DSS

	2	4	6	8	10	12 (days)[a]
control group	94.3±1.4]*	89.9±1.3]*	88.7±1.2]*	83.2±2.6]*	70.5±2.5]*	66.9±3.5]*
tryptophan group	96.5±0.7]	94.9±1.2]	94.6±1.7]	94.1±2.2]	90.1±2.5]	88.6±2.3]

*p<0.01 DSS: Dextran sulfate sodium
[a]days after administration of DSS
Body weight after DSS administration is given as a percentage of the basal body weight before DSS administration, taken as 100%.

Table 2. Frequencies of the bloody stool after administration of DSS

	2	4	6	8	10	12 (days)[a]
control group	9/10 (90%)]*	10/10 (100%)]*	10/10 (100%)]*	10/10 (100%)]*	10/10 (100%)]*	10/10 (100%)]*
tryptophan group	0/10 (0%)]	0/10 (0%)]	0/10 (0%)]	0/10 (0%)]	1/10 (10%)]	1/10 (10%)]

*p<0.01 DSS: Dextran sulfate sodium
[a]days after administration of DSS

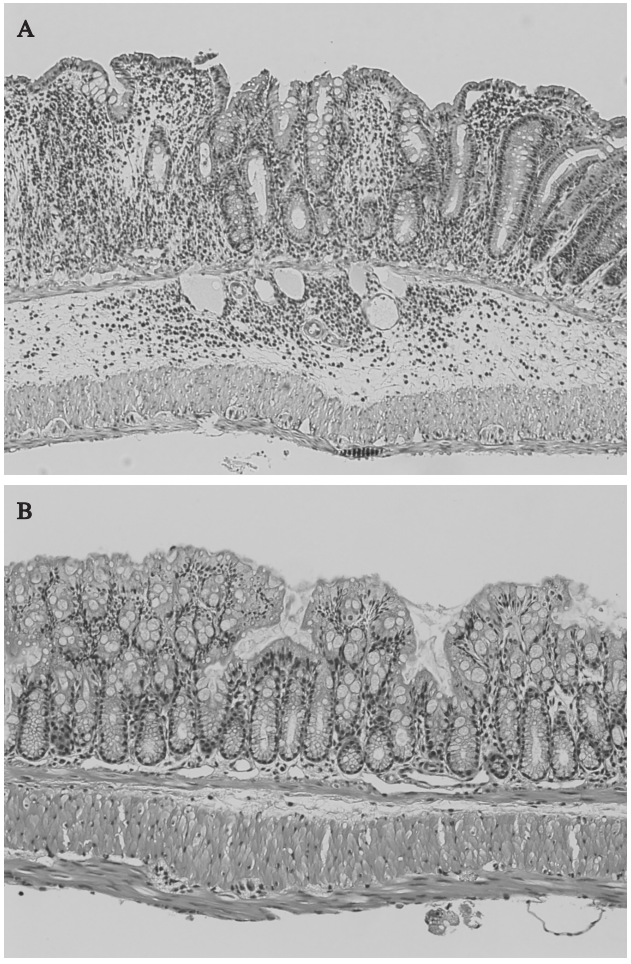

Figure 1. Histological findings of resected colon
HE staining of resected colons revealed abrasions of epithelium, cryptal disturbance, and inflammatory cell infiltration in mucosa and submucosal areas of colon in the control group. The tryptophan treatment remarkably attenuated these changes in comparison to the control group (A; the control group, B; the tryptophan group).

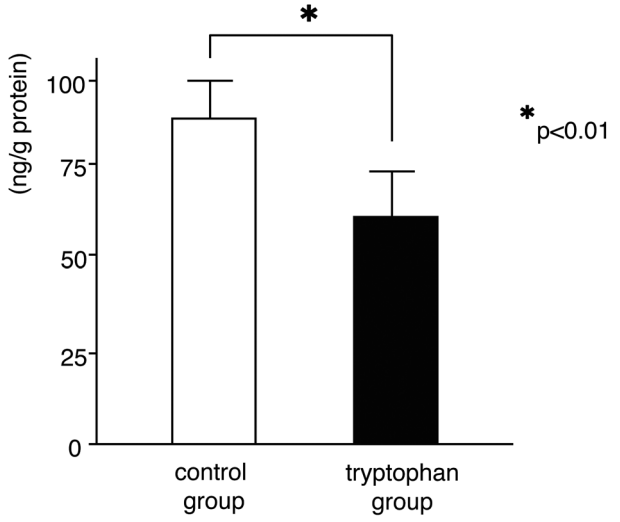

Figure 2. Nitrotyrosine levels in the resected colonic tissues
The tryptophan treatment (61.1±11.0) reduced significantly ($p<0.01$) nitrotyrosine levels in the resected colonic tissues in comparison to the control group (87.8±7.4) .

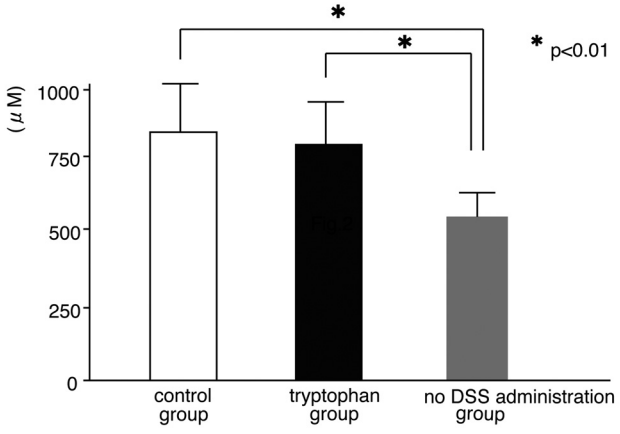

Figure 3. NOx levels among the three groups
NOx levels in urine (μM) in the (DSS-induced) control group and the tryptophan group were significantly increased compared with the control group without DSS administration. However, there was no significant difference between the (DSS-induced) control group and the tryptophan group.

nitrite, catalyzed by myeloperoxidase (MPO) (10). Therefore, tryptophan or its metabolites are estimated to suppress the either pathway, or both pathways. On the other hand, NOx is good indices of generation of NO, induced by NO synthase (NOS). Since reduction of NOx level by tryptophan treatment was not shown in this study, estimated mechanism of reduction of nitrotyrosine generation is not via suppression of NO and nitrite.

Therefore, tryptophan or its metabolites are estimated to suppress the generation of superoxide or MPO because theoretically, reduction of nitrotyrosine is via whether suppression of reaction of superoxide and NO or MPO and nitrite Superoxide, or its products may play roles in amelioration of colitis and reduction of nitrotyrosine level in this study. Excessive generation of superoxide is cytotoxic. Moreover, nitrotyrosine generation is via nitration of tyrosine residues by peroxynitrite which has cytotoxicity and induces severe tissue damage (9,10). Since peroxynitrite is generated by rapid reaction of superoxide and NO, suppression of superoxide generation may be potent mechanism of anti-colitis effect of tryptophan treatment. Still over, superoxide is generally catabolized hydrogen pero-

xide (H_2O_2) by superoxide dismutase (SOD). H_2O_2 is induced generation of hydroxyl radical which has strong oxidative effect and tissue damage under the some conditions such as Fenton's reaction. Therefore, suppression of superoxide is estimated to inhibit of generation of peroxynitrite or hydroxyl radical, followed by amelioration of colitis and reduction of nitrotyrosine generation.

Cytotoxicity of MPO itself has been discussable (11). In this study, tryptophan treatment remarkably ameliorated inflammatory cell infiltration of the colon. Since MPO is mainly included in neutrophils, possibility that tryptophan or its metabolites reduce the release of MPO from neutrophils is undeniable. Therefore, suppression of MPO is undeniable as possibile mechanism of suppression of nitrotyrosine although anti-colitis effects of MPO are unknown at present.

Tryptophan is metabolized via several pathways. Main are kynurenine pathway in human. 3-Hydroxyanthranilic acid (3-HA) and 3-hydroxykynurenine (3-HK) are metabolized via kynurenine pathway. However, these metabolites have been reported rather cofactors in the oxidative damage (12, 13). Another metabolite, 5-hydrotryptamine (5-HT), was reported to be involved in the pathogenesis of inflammation in experimental colitis (14). Moreover, 5-HT have been reported to accelerate NO production via endothelial NOS (eNOS) in vitro (15).

On the other hand, indole derivatives via kynurenine pathway have been reported to have anti-oxidative effects as scavengers of peroxynitrite (16). Moreover, serotonin, 5-hydroxytryptophan (5-HTP), N-acetylserotonin (NAS), and melatonin which are metabolized via serotonin pathway have been reported to have several anti-oxidative effects (17-20). Serotonin, NAS, melatonin have been reported to have suppressive effects of superoxide generation. Still over, 5-HTP, precursor of serotonin has been reported to reduce the oxidative damage by suppression of ROS or peroxynitrite (21). Nevertheless melatonin is directly scavengers a variety of free radicals and an indirect antioxidant (22), a contribution of melatonin can be ruled out in the present model because C57black6 mice do not synthesize melatonin.

Therefore, we hypothesized that metabolites such as indole derivatives, serotonin, 5-HTP and NAS may be candidates to play key roles of suppression of superoxide or peroxynitrite, and ameliorated DSS-induced colitis and reduced nitrotyrosine level in this study.

In conclusion, our study indicate that tryptophan treatment ameliorated DSS-induced colitis, and one of the anti-colitis mechanism may be anti-oxidative or nitration stress although contributions of these compounds to the protective effect of tryptophan remain to be addressed.

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