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ORIGINAL ARTICLE

Melatonin alters amino acid metabolism and inflammatory responses in colitis mice

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Abstract Inflammatory bowel disease is a chronic inflammatory dysfunction of the gastrointestinal tract. This study explored the hypothesis that melatonin has beneficial functions in the mouse model of colitis induced by dextran sodium sulfate (DSS), with a specific focus on the expression of intestinal inflammatory cytokines and the serum levels of amino acids. The results revealed that mice with melatonin supplementation had a reduction in weight loss and disease index induced by DSS treatment. Melatonin stifled the expression of colonic IL-17 in mice with DSS-induced colitis. Melatonin also lowered the serum levels of Asp, Ser, Met, and Leu ($p < 0.05$), but increased those of Glu and Cys ($p < 0.05$). Thus, melatonin treatment is promising and may function as a potential adjuvant therapy to alleviate the clinical symptoms of patients with inflammatory bowel disease.

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Keywords Inflammatory bowel disease · Melatonin · IL-17 · TNF-α · Glutamine

Abbreviations

IBD	Inflammatory bowel disease
DSS	Dextran sodium sulfate
UC	Ulcerative colitis
CD	Crohn's disease
GIT	Gastrointestinal tract

Introduction

Inflammatory bowel disease (IBD), such as Crohn's disease (CD) and ulcerative colitis (UC), affects the gastrointestinal tract (GIT) and is characterized by recurrent diarrhea, abdominal pain and discomfort, and weight loss. Various environmental, genetic, and host factors contribute to the development of IBD (Vora et al. 2012), which is a long-term condition with the potential to severely degrade the quality of life. There are more people in Western nations with this disease, although doctors are starting to see an increase in the number of diagnoses across Asia (Engel and Neurath 2010). The development of IBD is characterized by the expression of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-17, and the activation of inflammatory signaling, such as nuclear factor kappa B (NF-κB) (Algieri et al. 2013; Trivedi and Jena 2012). An increase in intestinal permeability is another indicator for the development of IBD (Toedter et al. 2012). A suitable anti-inflammatory may help to alleviate the symptoms of IBD and minimize the damage to the intestinal tract.

Another indicator for the development of IBD is the alteration of amino acid metabolism. For example, an early

investigation showed that hyperhomocysteinemia is more common in IBD patients than in healthy controls, suggesting a possible pathogenetic link between IBD and amino acid metabolism (Romagnuolo et al. 2001). Indeed, in our previous study using a mouse model, we also found a significant alteration in the serum levels of amino acids with the development of IBD (Ren et al. 2014c; Liu et al. 2017). Three days after DSS treatment, a lower serum level of tryptophan and a higher serum level of glutamine were observed. Seven days after DSS treatment, the serum levels of tryptophan, γ-amino-*n*-butyric acid, glutamic acid, and aspartic acid had decreased. Further studies have demonstrated that the development and progression of IBD can be significantly shaped by dietary supplementation with amino acids, such as L-tryptophan (Kim et al. 2010), L-cysteine (Kim et al. 2009), L-arginine (Ren et al. 2014c; Coburn et al. 2012), and L-glutamine (Ren et al. 2014c; Crespo et al. 2012). These compelling investigations suggest that amino acid metabolism greatly influences the etiology of IBD.

There is increasing evidence to suggest that melatonin has anti-inflammatory properties and may reduce the symptoms of colitis (Esposito and Cuzzocrea 2010; Spreer et al. 2006; Mauriz et al. 2013; Ren et al. 2017a). Melatonin is a potent antioxidant that is produced by various tissues and supports the management of circadian rhythms (Marquez et al. 2006). As it exists in large quantities in the gastrointestinal tract, it has the potential to influence its health and function in a substantial way (Nosal'ova et al. 2007; Ren et al. 2017b). For example, previous study found that melatonin improves the intestinal morphology in the weanling mice (Ren et al. 2017c). The current study aimed to explore the effects of melatonin in mice with DSS-induced colitis, focusing on the expression of inflammatory cytokines and the serum levels of amino acids.

Materials and methods

Animals and experimental design

Thirty 8-week-old mice, each weighing approximately 23 g, were used in this study. With a completely randomized design, the mice were allotted to three different treatment groups: the CTRL group, fed a basal diet (Ren et al. 2014a and b) and normal water; the DSS group, with basal diet and water with 5% DSS; and the MELDSS group, with basal diet and water with 5% DSS and 0.2 mg/mL melatonin. The mice were housed at a temperature of 22–24 °C and a humidity level of 55–60% with a 12-h light/dark rotation (lights activated at 08:00). All mice were administered a basal diet for 3 days prior to the start of the study. For the following 7 days, the animals were permitted to eat and drink freely. On day 8, the mice were weighed to determine the average amount of weight gain in each day. They were then humanely terminated and samples of blood were taken as previous report (Liu et al. 2017). Colon samples were collected for histological analysis or frozen in liquid nitrogen for gene expression analysis. Our experimental design was conducted with the full approval of the Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. All methods and practical steps were in line with animal welfare rules in China.

Histological analysis

Histological grading of colitis symptoms was performed according to our previous report (Liu et al. 2017). Briefly, after fixing the colon tissue in 10% formalin, hematoxylin and eosin staining (H&E staining) was performed to allow histological characterization of the colitis (Varshney et al. 2013; Liu et al. 2017). Lesions, including inflammation, edema, erosion, cryptitis, ulcers, and goblet cell hyperplasia, were scored using a scale of zero to four. A score of zero indicated no colitis or epithelial thickening; a score of one indicated an increased number of mucosal leukocytes and/or slight epithelial cell hyperplasia; a score of two indicated multiple loci of inflammation, leukocytic mucosal and submucosal infiltration, and/or marked epithelial cell hyperplasia; a score of three indicated extensive mucosal and submucosal leukocytes, depletion of mucin-secreting goblet cells, and/or pronounced epithelial cell hyperplasia; and a score of four indicated extensive transmural leukocytic infiltration, crypt abscesses, and/or marked epithelial cell hyperplasia (Liu et al. 2017).

Serum amino acid analysis

The serum from the blood samples was collected via centrifugation at 2000 rpm for 10 min at 4 °C. Sulfosalicylic acid (2.7 mL) and the supernatant (300 μL) were added to a sealed centrifuge container. The two substances were shaken together and allowed to rest for 15 min before being centrifuged at 1000 rpm for 10 min at 4 °C. Following this, the supernatant was filtered through a 0.45-μm membrane and analyzed using a Hitachi L-8900 automatic amino acid analyzer.

RT-PCR

The total RNA was extratced from the colon tissues according to methods described in previous reports (Ren et al. 2016a; Ren et al. 2017d). The RNA was treated with DNase I (Invitrogen, USA) according to the manufacturer's instructions. The amount of total RNA in the samples was determined spectrophotometrically at 260 nm. The amplification reactions were conducted using an ABI

Prism 7900HT Sequence Detection System. The relative levels of genes were calculated according to the $2^{-(\Delta\Delta C_t)}$ method, where $Ct = (Ct_{target} - Ct_{\beta\text{-actin}})_{treatment} - (Ct_{target} - Ct_{\beta\text{-actin}})_{control}$. The primers used in this study are summarized in Table 1.

Statistical analysis

The statistical analysis was conducted using the SPSS 22.0 software. The data are expressed as the mean ± standard error of the mean (SEM). Values in the same row with different superscripts are considered to be significantly different ($p < 0.05$), whereas values with the same superscripts are considered to be not significantly different ($p > 0.05$). Collective comparisons were carried out by ANOVA (one-way analysis of variance) with Levene's test and Tukey's multiple comparison test.

Results

In terms of the diagnosis of IBD, clinicians place a major emphasis on weight loss. For the DSS mice, the results showed a substantially greater degree of weight loss ($p < 0.05$). These mice were unable to gain weight in the same way as the CTRL mice. However, treatment with melatonin appeared to alleviate this process ($p < 0.05$) (Fig. 1A). Furthermore, mice in DSS group were observed to exhibit substantially more colitis symptoms than the CTRL mice and the MELDSS mice (Fig. 1B).

Table 2 shows the analysis of the serum levels of amino acids. For the DSS mice, there was a substantial increase in the level of Gln and decrease in the levels of His and Phe ($p < 0.05$) relative to the control mice. Meanwhile, for the MELDSS mice, the levels of Asp, Ser, Met, and Leu decreased substantially ($p < 0.05$), but those of Gln and Cys rose by a significant amount ($p < 0.05$) in comparison to the DSS mice.

Table 1 Primers used in this study

Primers	GenBank access number	Sequence of primer	Product length (bp)
IL-4	NM_021283.2	F: GAGCCATATCCACGGATGCGACAA R: CATGGTGGCTCAGTACTACGAGTA	382
IL-10	XM_021175612.1	F: GCCACATGCTCCTAGAGCTG R: CAGCTGGTCCTTTGTTTGAAA	71
TNF-α	XM_021218154.1	F: AGGCACTCCCCCAAAAGAT R: TGAGGGTCTGGGCCATAGAA	192
IL-17	NM_010552.3	F: TACCTCAACCGTTCCACGTC R: TTTCCCTCCGCATTGACAC	119
IL-18	XM_006510028.3	F: AGACAACTTTGGCCGACTTC R: CCTTCACAGAGAGGGTCACA	203
IL-22	XM_006513865.3	F: GCTCAGCTCCTGTCACATCA R: CACTGTCTCCTTCAGCCTTCT	73
IL-23	NM_031252.2	F: AGTGTGAAGATGGTTGTGAC R: CTGGAGGAGTTGGCTGAG	194
Tlr4	XM_006509283.3	F: TTCAGAACTTCAGTGGCTGGATT R: CCATGCCTTGTCTTCAATTGTTT	64
β-Actin	NM_007393.5	F: AACGAGCGGTTCCGATGC R: GTAGTTCATGGATGCCACAGG	79

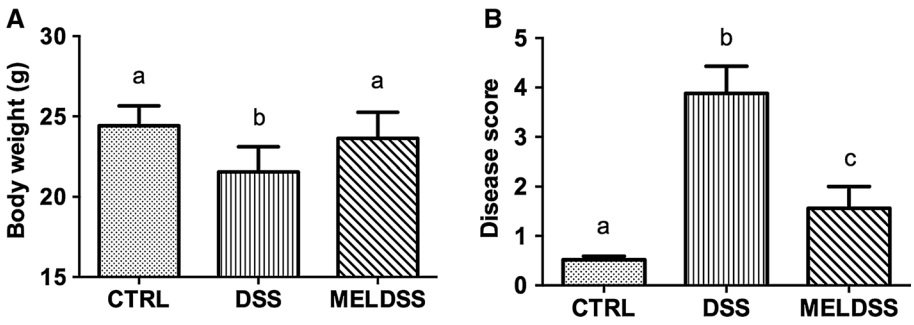

Fig. 1 Impact of melatonin treatment on **A** the body weight and **B** the disease score

Table 2 Effect of melatonin on the serum amino acids (μg/mL)

	CON group	DSS group	MEL group	*p* value
Asp	3.18 ± 0.07	3.58 ± 0.19	2.48 ± 0.22[d]	0.004
Gln	30.04 ± 0.56	36.21 ± 0.71[b]	40.88 ± 1.84[c]	<0.001
Lys	88.35 ± 4.42	99.98 ± 6.77	93.46 ± 2.09	0.181
His	14.16 ± 0.54	12.52 ± 0.41[a]	12.46 ± 0.64	0.037
Arg	40.68 ± 1.3	44.56 ± 3.39	41.91 ± 0.84	0.363
Gly	23.58 ± 1.18	24.64 ± 1.27	24.82 ± 1.25	0.554
Ser	23.74 ± 1.97	24.19 ± 1.44	18.78 ± 1.32[c]	0.018
Cys	3 ± 0.34	2.94 ± 0.29	4.09 ± 0.37[c]	0.035
Thr	91.8 ± 1.85	93.38 ± 3.6	95.08 ± 3.36	0.705
Tyr	11.95 ± 0.52	12.11 ± 0.57	12.08 ± 0.58	0.845
Ala	46.09 ± 0.91	46.18 ± 3.37	44.46 ± 3.13	0.716
Phe	17.28 ± 0.17	15.24 ± 0.23[b]	14.7 ± 0.36	<0.001
Val	24.88 ± 1.04	26.13 ± 0.85	25.22 ± 0.88	0.396
Met	18.62 ± 1.37	18.03 ± 1.35	13.85 ± 0.65[c]	0.014
Ile	12.06 ± 0.55	12.88 ± 0.32	11.71 ± 0.65	0.136
Leu	18.52 ± 0.76	19.63 ± 0.4	17.29 ± 0.67[c]	0.015
Trp	22.43 ± 0.28	21.64 ± 0.59	20.22 ± 0.45	0.086
Pro	12.5 ± 0.8	9.9 ± 1.26	10.35 ± 0.89	0.114

[a] The amino acids in DSS group differ from the CTRL group with $p < 0.05$

[b] The amino acids in DSS group differ from the CTRL group with $p < 0.01$

[c] The amino acids in MELDSS group differ from the DSS group with $p < 0.05$

[d] The amino acids in MELDSS group differ from the DSS group with $p < 0.01$

No significant differences were observed in the levels of IL-18, IL-23, IL-4, TLR4, or IL-22 (Fig. 2). Unlike the DSS mice, a substantial reduction in TNF-α ($p < 0.05$) was found for the MELDSS mice (Fig. 2). Furthermore, the level of IL-17 increased by a significant amount for the DSS mice ($p < 0.05$) in comparison with the CTRL mice. However, upon the treatment with melatonin, this increased level of IL-17 was interrupted and controlled ($p < 0.05$) (Fig. 2).

Discussion

IBD in human patients is defined by recurrent episodes of acute inflammation within the gastrointestinal tract. This can cause severe discomfort, which is one of the hardest symptoms for patients with this disease to deal with (Lobaton et al. 2014; Gasparetto and Guariso 2013). In this study, 5% DSS was used to trigger the development of acute colitis in mice and explore the effects of melatonin on DSS-induced colitis. The outcomes appear to support the idea that DSS-induced colitis leads to increased weight loss.

According to the results of our study, Gln was more abundant in DSS mice than in control mice ($p < 0.05$). However, MELDSS mice exhibited higher levels of Gln than DSS mice ($p < 0.05$). In a previous experiment, we discovered that the profile of serum amino acids is altered in subjects with DSS-induced colitis (Ren et al. 2014c). Crucially, the prescription of glutamine treatments appears to be an effective method for increasing biochemical and clinical functions. This involves the activation of the myosin light chain kinase (MLCK), NF-κB, and PI3K-Akt signaling channels. This phenomenon was tested in a murine colitis model of injury and repair, but the outcomes were similar to those of human subjects (Ren et al. 2014c). The presence of rising Gln volumes in the DSS mice suggests that, for IBD sufferers, the substance can be used to alleviate inflammatory responses and enhance the health of metabolic tissues. Within the intracellular pool and mammalian plasma systems, Gln is the most abundant free amino acid and performs a number of functions relating to physical health and wellbeing. For instance, it is essential for the proliferative regulation of rapidly dividing immune cells and enterocytes (Altman et al. 2016).

Much evidence has been presented to demonstrate that melatonin has powerful anti-inflammatory, anti-apoptotic, and antioxidant qualities (Trivedi and Jena 2013; Chung et al. 2014), and as such, it may hold the key to more successful IBD treatments. Melatonin plays a number of vital neurohormonal functions. As it is closely related to serotonin, it suppresses the smooth muscle cell contractions and detrimental gastrointestinal activity triggered by serotonin. It is not completely clear why melatonin is present in the GIT at such abundant levels. The levels of melatonin in the GIT are usually around 400-fold greater than those in the pineal gland (Thor et al. 2007). We do know that it is manufactured in the enterochromaffin cells that make up the gut. Studies have also shown that it cannot be created without the contribution of L-tryptophan (Chen et al. 2011). According to our earlier research, the influence of melatonin on inflammation is significant and, therefore, melatonin should be considered a potential treatment for IBD and similar conditions (Trivedi and Jena 2013; Motilva et al. 2011). For some time, clinicians have used corticosteroids, immunosuppressants, biological agents, and aminosalicylates to treat the symptoms of IBD (Bressler et al. 2015). However, they are also in the process of examining many other potential treatments, such as probiotics, new biologics, prebiotics, and peroxisome proliferator-activated receptor γ agents (Shen et al. 2014b; Shen et al. 2014a).

The contribution of cytokines is significant, particularly when it comes to the immunopathogenesis of IBD. It has been shown that the administration of DSS resulted in the production of pro-inflammatory cytokines in and around the diseased region (Beloqui et al. 2013). These authors discovered that treatment with melatonin actually attenuates the increase of IL-17 within the jejunum. The induced Th17

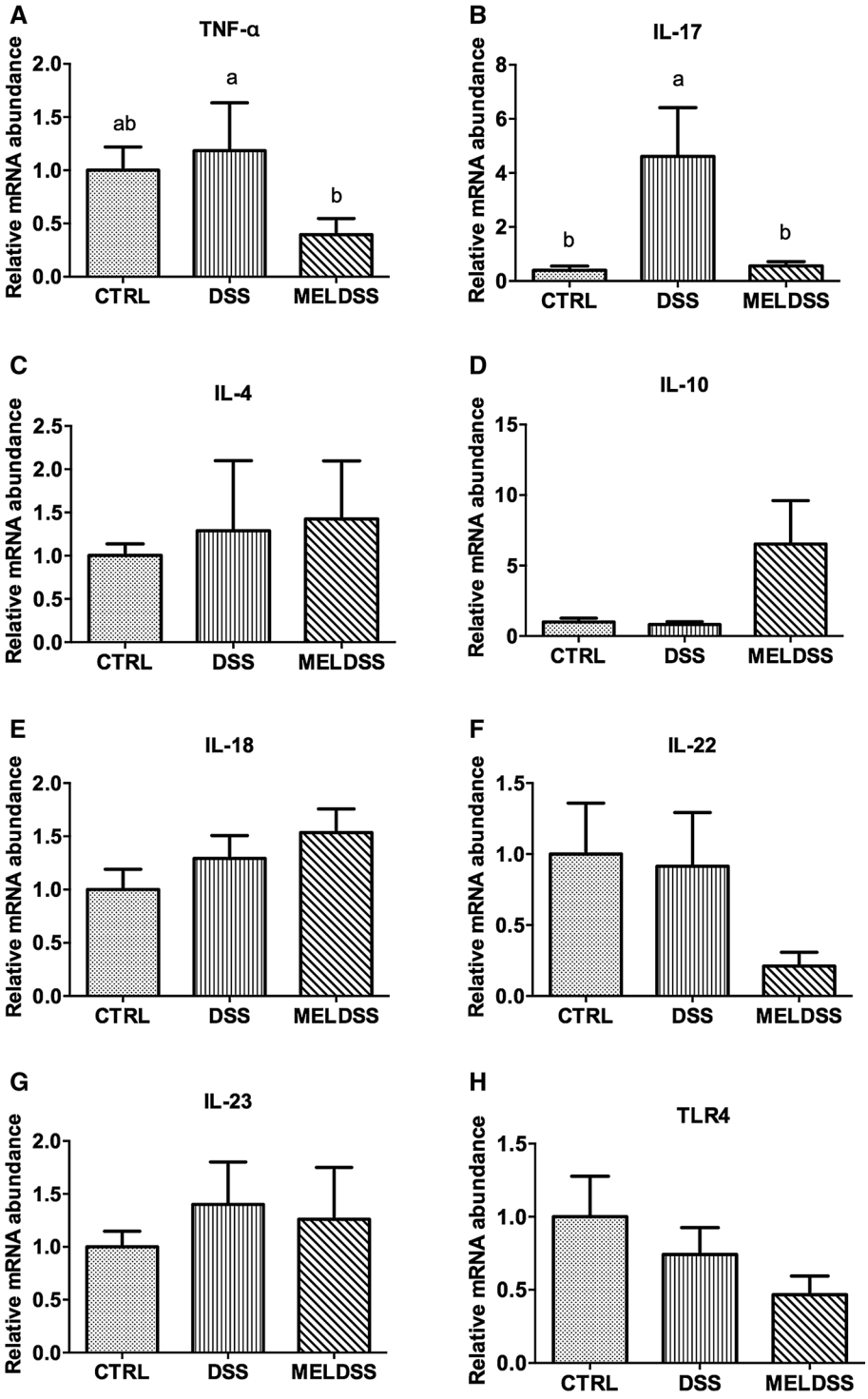

Fig. 2 Impact of melatonin treatment on the expressions of colonic inflammatory cytokines. The relative gene expression level determined by RT-PCR was shown in **A** (TNF-α), **B** (IL-17), **C** (IL-4), **D** (IL-10), **E** (IL-18), **F** (IL-22), **G** (IL-23), and **H** (TLR4)

(iTh17) and natural Th17 (nTh17) cells are responsible for creating IL-17 (Ren et al. 2016b; Ren et al. 2017b). IL-17 supports the migration of monocytes and neutrophils via chemokine processes to the damaged areas of the body. This is considered to have an important influence on how autoimmune conditions emerge and develop (Koga et al. 2014). Our results indicate that treatment with melatonin is an effective way to reduce IL-17 levels and, consequently, alleviate the

symptoms of chronic IBD (where they involve the contribution of IL-17 as part of their original progression). For IBD patients, substantial levels of pro-inflammatory cytokines are normally observed. These include IL-6, TNF-α, and IL-1β (Reimund et al. 1996). However, it should be noted that, in the present study, the DSS mice did not exhibit higher levels of pro-inflammatory cytokines (IL-1β) or higher than usual colonic activity. The level of TNF-α did rise by a small amount ($p > 0.05$). TNF-α is a pleiotropic cytokine that contributes to various inflammatory functions and is closely connected to the development of IBD. It has been demonstrated to influence the stimulation of cytotoxic, acute, and apoptotic phase reactions, adhesion molecule activities, and increases in intestinal permeability (Hyam et al. 2013). For this reason, melatonin has the potential to serve as a long-term immunoregulator and anti-inflammatory compound.

In summary, this study has revealed that melatonin treatment is an effective way to control and alleviate IBD symptoms and regulate inflammatory processes. Furthermore, melatonin highly shapes the serum levels of amino acids. These results indicate that melatonin is a valuable form of adjuvant treatment for patients suffering from IBD. In spite of this, additional research is needed to confirm this connection and further elucidate the relationships between melatonin and the GI tract and other parts of the body.

Author contributions GL, QJ, JF, WR, and YY conceived the experiment(s); GL, QJ, WR, SC, JY, and KY conducted the experiments; GL and SC analyzed the results. GL and WR prepared the manuscript. All authors reviewed the manuscript.

Compliance with ethical standards

Ethical standards The protocol for this study was approved by the Committee on the Ethics of Animal Experiments of Institute of Subtropical Agriculture, Chinese Academy of Sciences (Permit Number: 201602-05) and it was conducted out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Institute of Subtropical Agriculture, Chinese Academy of Sciences.

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Conflict of interest All co-authors have seen and agreed with the contents of the manuscript and there is no financial interest to report.

References

Algieri F, Zorrilla P, Rodriguez-Nogales A, Garrido-Mesa N, Banuelos O, Gonzalez-Tejero MR, Casares-Porcel M, Molero-Mesa J, Zarzuelo A, Utrilla MP, Rodriguez-Cabezas ME, Galvez J (2013) Intestinal anti-inflammatory activity of hydroalcoholic extracts of *Phlomis purpurea* L. and *Phlomis lychnitis* L. in the trinitrobenzenesulphonic acid model of rat colitis. J Ethnopharmacol 146(3):750–759. doi:10.1016/j.jep.2013.01.041

Altman BJ, Stine ZE, Dang CV (2016) From Krebs to clinic: glutamine metabolism to cancer therapy. Nat Rev Cancer 16(10):619–634. doi:10.1038/nrc.2016.71

Beloqui A, Coco R, Alhouayek M, Solinis MA, Rodriguez-Gascon A, Muccioli GG, Preat V (2013) Budesonide-loaded nanostructured lipid carriers reduce inflammation in murine DSS-induced colitis. Int J Pharm 454(2):775–783. doi:10.1016/j.ijpharm.2013.05.017

Bressler B, Marshall JK, Bernstein CN, Bitton A, Jones J, Leontiadis GI, Panaccione R, Steinhart AH, Tse F, Feagan B, Colitis TU (2015) Clinical practice guidelines for the medical management of nonhospitalized ulcerative colitis: the Toronto consensus. Gastroenterology 148(5):1035–1058. doi:10.1053/j.gastro.2015.03.001

Chen CQ, Fichna J, Bashashati M, Li YY, Storr M (2011) Distribution, function and physiological role of melatonin in the lower gut. World J Gastroenterol 17(34):3888–3898. doi:10.3748/wjg.v17.i34.3888

Chung SH, Park YS, Kim OS, Kim JH, Baik HW, Hong YO, Kim SS, Shin JH, Jun JH, Jo Y, Ahn SB, Jo YK, Son BK, Kim SH (2014) Melatonin attenuates dextran sodium sulfate induced colitis with sleep deprivation: possible mechanism by microarray analysis. Dig Dis Sci 59(6):1134–1141. doi:10.1007/s10620-013-3013-2

Coburn LA, Gong X, Singh K, Asim M, Scull BP, Allaman MM, Williams CS, Rosen MJ, Washington MK, Barry DP, Piazuelo MB, Casero RA, Chaturvedi R, Zhao ZM, Wilson KT (2012) L-Arginine supplementation improves responses to injury and inflammation in dextran sulfate sodium colitis. PLoS One 7(3):e33546. doi:10.1371/journal.pone.0033546

Crespo I, San-Miguel B, Prause C, Marroni N, Cuevas MJ, Gonzalez-Gallego J, Tunon MJ (2012) Glutamine treatment attenuates endoplasmic reticulum stress and apoptosis in TNBS-induced colitis. PLoS One 7(11):e50407. doi:10.1371/journal.pone.0050407

Engel MA, Neurath MF (2010) New pathophysiological insights and modern treatment of IBD. J Gastroenterol 45(6):571–583. doi:10.1007/s00535-010-0219-3

Esposito E, Cuzzocrea S (2010) Antiinflammatory activity of melatonin in central nervous system. Curr Neuropharmacol 8(3):228–242

Gasparetto M, Guariso G (2013) Highlights in IBD epidemiology and its natural history in the paediatric age. Gastroenterol Res Pract 2013:829040. doi:10.1155/2013/829040

Hyam SR, Jang SE, Jeong JJ, Joh EH, Han MJ, Kim DH (2013) Echinocystic acid, a metabolite of lancemaside A, inhibits TNBS-induced colitis in mice. Int Immunopharmacol 15(2):433–441. doi:10.1016/j.intimp.2012.12.017

Kim CJ, Kovacs-Nolan J, Yang C, Archbold T, Fan MZ, Mine Y (2009) L-Cysteine supplementation attenuates local inflammation and restores gut homeostasis in a porcine model of colitis. BBA-Gen Subj 1790(10):1161–1169. doi:10.1016/j.bbagen.2009.05.018

Kim CJ, Kovacs-Nolan JA, Yang CB, Archbold T, Fan MZ, Mine Y (2010) L-Tryptophan exhibits therapeutic function in a porcine model of dextran sodium sulfate (DSS)-induced colitis. J Nutr Biochem 21(6):468–475. doi:10.1016/j.jnutbio.2009.01.019

Koga T, Hedrich CM, Mizui M, Yoshida N, Otomo K, Lieberman LA, Rauen T, Crispin JC, Tsokos GC (2014) CaMK4-dependent activation of AKT/mTOR and CREM-alpha underlies autoimmunity-associated Th17 imbalance. J Clin Investig 124(5):2234–2245. doi:10.1172/Jci73411

Liu G, Yu L, Fang J, Hu CA, Yin J, Ni H, Ren W, Duraipandiyan V, Chen S, Al-Dhabi NA, Yin Y (2017) Methionine restriction on oxidative stress and immune response in dss-induced colitis mice. Oncotarget 8(27):44511–44520. doi:10.18632/oncotarget.17812

Lobaton T, Vermeire S, Van Assche G, Rutgeerts P (2014) Review article: anti-adhesion therapies for inflammatory bowel disease. Aliment Pharmacol Ther 39(6):579–594. doi:10.1111/apt.12639

Marquez E, Sanchez-Fidalgo S, Calvo JR, la de Lastra CA, Motilva V (2006) Acutely administered melatonin is beneficial while chronic melatonin treatment aggravates the evolution of TNBS-induced colitis. J Pineal Res 40(1):48–55. doi:10.1111/j.1600-079X.2005.00275.x

Mauriz JL, Collado PS, Veneroso C, Reiter RJ, Gonzalez-Gallego J (2013) A review of the molecular aspects of melatonin's anti-inflammatory actions: recent insights and new perspectives. J Pineal Res 54(1):1–14. doi:10.1111/j.1600-079X.2012.01014.x

Motilva V, Garcia-Maurino S, Talero E, Illanes M (2011) New paradigms in chronic intestinal inflammation and colon cancer: role of melatonin. J Pineal Res 51(1):44–60. doi:10.1111/j.1600-079X.2011.00915.x

Nosal'ova V, Zeman M, Cerna S, Navarova J, Zakalova M (2007) Protective effect of melatonin in acetic acid induced colitis in rats. J Pineal Res 42(4):364–370. doi:10.1111/j.1600-079X.2007.00428.x

Reimund JM, Wittersheim C, Dumont S, Muller CD, Baumann R, Poindron P, Duclos B (1996) Mucosal inflammatory cytokine production by intestinal biopsies in patients with ulcerative colitis and Crohn's disease. J Clin Immunol 16(3):144–150

Ren WK, Chen S, Yin J, Duan JL, Li TJ, Liu G, Feng ZM, Tan BE, Yin YL, Wu GY (2014a) Dietary arginine supplementation of mice alters the microbial population and activates intestinal innate immunity. J Nutr 144(6):988–995. doi:10.3945/jn.114.192120

Ren WK, Duan Jl, Yin J, Liu G, Cao Z, Xiong X, Chen S, Li TJ, Yin YL, Hou YQ, Wu GY (2014b) Dietary L-glutamine supplementation modulates microbial community and activates innate immunity in the mouse intestine. Amino Acids 46(10):2403–2413. doi:10.1007/s00726-014-1793-0

Ren WK, Yin J, Wu MM, Liu G, Yang G, Xion Y, Su DD, Wu L, Li TJ, Chen S, Duan JL, Yin YL, Wu GY (2014c) Serum amino acids profile and the beneficial effects of l-arginine or l-glutamine supplementation in dextran sulfate sodium colitis. PLoS One 9(2):e88335. doi:10.1371/journal.pone.0088335

Ren WK, Yin J, Chen S, Duan JL, Liu G, Li TJ, Li NZ, Peng YY, Tan BE, Yin YL (2016a) Proteome analysis for the global proteins in the jejunum tissues of enterotoxigenic Escherichia coli -infected piglets. Sci Rep 6:25640. doi:10.1038/srep25640

Ren WK, Yin J, Duan JL, Liu G, Tan BE, Yang G, Wu GY, Bazer FW, Peng YY, Yin YL (2016b) mTORC1 signaling and IL-17 expression: defining pathways and possible therapeutic targets. Eur J Immunol 46(2):291–299. doi:10.1002/eji.201545886

Ren WK, Liu G, Chen S, Yin J, Wang J, Tan B, Wu G, Bazer FW, Peng YY, Li TJ, Reiter RJ, Yin YL (2017a) Melatonin signaling in T cells: functions and applications. J Pineal Res 62(3):e12394. doi:10.1111/jpi.12394

Ren WK, Liu G, Yin J, Tan B, Wu G, Bazer FW, Peng YY, Yin YL (2017b) Amino-acid transporters in T-cell activation and differentiation. Cell Death Dis 8(5):e2757. doi:10.1038/cddis.2017.207

Ren WK, Wang P, Yan JM, Liu G, Zeng BH, Hussain T, Peng C, Yin J, Tan B, Li TJ, Wei H, Zhu GQ, Reiter RJ, Yin YL (2017c) Melatonin alleviates weanling stress in mice: involvement of intestinal microbiota. J Pineal Res. doi:10.1111/jpi.12448

Ren WK, Yin J, Xiao H, Chen S, Liu G, Tan B, Li NZ, Peng YY, Li TJ, Zeng BH, Li WX, Wei H, Yin ZN, Wu GY, Hardwidge PR, Yin YL (2017d) Intestinal microbiota-derived GABA mediates interleukin-17 expression during enterotoxigenic escherichia coli Infection. Front Immunol 7:685. doi:10.3389/fimmu.2016.00685

Romagnuolo J, Fedorak RN, Dias VC, Bamforth F, Teltscher M (2001) Hyperhomocysteinemia and inflammatory bowel disease: prevalence and predictors in a cross-sectional study. Am J Gastroenterol 96(7):2143–2149. doi:10.1016/S0002-9270(01)02513-8

Shen J, Zuo ZX, Mao AP (2014a) Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials. Inflamm Bowel Dis 20(1):21–35. doi:10.1097/01.MIB.0000437495.30052.be

Shen J, Zuo ZX, Mao AP (2014b) Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials. Inflamm Bowel Dis 20(12):2526–2528. doi:10.1097/Mib.0000000000000254

Spreer A, Gerber J, Baake D, Hanssen M, Huether G, Nau R (2006) Antiinflammatory but no neuroprotective effects of melatonin under clinical treatment conditions in rabbit models of bacterial meningitis. J Neurosci Res 84(7):1575–1579. doi:10.1002/jnr.21055

Thor PJ, Krolczyk G, Gil K, Zurowski D, Nowak L (2007) Melatonin and serotonin effects on gastrointestinal motility. J Physiol Pharmacol 58(Suppl 6):97–103

Toedter G, Li K, Sague S, Ma KY, Marano C, Macoritto M, Park J, Deehan R, Matthews A, Wu GD, Lewis JD, Arijs I, Rutgeerts P, Baribaud F (2012) Genes associated with intestinal permeability in ulcerative colitis: changes in expression following infliximab therapy. Inflamm Bowel Dis 18(8):1399–1410. doi:10.1002/ibd.22853

Trivedi PP, Jena GB (2012) Dextran sulfate sodium-induced ulcerative colitis leads to increased hematopoiesis and induces both local as well as systemic genotoxicity in mice. Mutat Res 744(2):172–183. doi:10.1016/j.mrgentox.2012.03.001

Trivedi PP, Jena GB (2013) Melatonin reduces ulcerative colitis-associated local and systemic damage in mice: investigation on possible mechanisms. Dig Dis Sci 58(12):3460–3474. doi:10.1007/s10620-013-2831-6

Varshney J, Ooi JH, Jayarao BM, Albert I, Fisher J, Smith RL, Patterson AD, Cantorna MT (2013) White button mushrooms increase microbial diversity and accelerate the resolution of *Citrobacter rodentium* infection in mice. J Nutr 143(4):526–532

Vora P, Shih DQ, McGovern DP, Targan SR (2012) Current concepts on the immunopathogenesis of inflammatory bowel disease. Front Biosci (Elite Ed) 4:1451–1477