Melatonin and Ulcerative Colitis: Evidence, Biological Mechanisms, and Future Research

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Abstract: Ulcerative colitis (UC) is an inflammatory bowel disease that afflicts up to 1 million people in the US. Current treatments for UC are mostly nonspecific, not always effective, and often accompanied by serious side effects. Therefore, there is considerable interest in finding alternative and more tolerable treatments for this disease. Physiologic data suggest that melatonin is an important regulator of both inflammation and motility in the gastrointestinal tract, and data from in vitro studies, animal experiments, and limited studies in humans suggest that supplemental melatonin may have an ameliorative effect on colitis. In this review we summarize the evidence regarding melatonin as a possible therapeutic agent in UC and discuss possible biological mechanisms and directions for future research.

(*Inflamm Bowel Dis 2009;15:134–140)*

Key Words: melatonin, inflammatory bowel diseases, ulcerative colitis, clinical trials, animal models

ULCERATIVE COLITIS

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) that afflicts up to 1 million people in the US. UC is often a debilitating disease with considerable impact on quality of life. UC can be characterized as a diffuse, continuous, and superficial inflammation of the colon from the anorectal verge to a proximal extent that varies between individuals—left-sided colitis (inflammation up to the splenic flexure) or extensive colitis (inflammation beyond the splenic flexure).[1,2] Features relevant to diagnosis, in addition to those symptoms mentioned above, include family history, extraintestinal manifestations (e.g., joints, rashes, eyes), full blood count, liver function tests, erythrocyte sedimentation rate, C-reactive protein levels, urea, electrolytes, stool culture, *Clostridium difficile* toxin assessment, endoscopy, and biopsy.[3,4] Still, there appears to be no pathognomonic marker that can definitively diagnose UC, and no specific feature of the disease that can distinguish it from other specific forms of colitis.[5] Patients with UC may have histological features such as microscopic inflammation of the ileum, histological gastritis, periappendiceal inflammation, patchiness, and relative rectal sparing at the time of diagnosis.[6,7]

The prevalence of UC has increased in the past few decades in North America and Europe and is also becoming more common in other parts of the world.[8] UC can occur in people of any age, but it usually starts between the ages of 15 and 30. It affects men and women equally and appears associated with inheritable genetic traits, including specific HLA haplotypes.[9,10] A higher incidence of UC is seen in Whites and people of Jewish descent. Approximately 15% of patients with UC develop an acute attack of severe colitis, and 30% of these patients require colectomy. Patients with UC tend to have increased risks of various conditions, including anemia, other nutrient deficiencies, liver/gall bladder disease, kidney stones, osteoporosis, and colon cancer, depending on the duration and extent of the disease.[3,4]

The etiology and pathogenesis of UC remain unclear. In the broad sense, UC (and IBD in general) is believed to involve several factors, some biologically interrelated, including inflammation, oxidative stress, infection, neural autonomic, and other factors.[11–18] More specifically, the development of UC is thought to be the result of an exaggerated or insufficiently suppressed immune response to an undefined luminal antigen(s), probably derived from the microbial flora.[19] This inflammatory process leads to recruitment of proinflammatory T cells promoting chemoattraction of neutrophils and other mediators of antibacterial defense and inflammation, mucosal damage, and consequent further disturbance of the epithelial barrier function, thus exacerbating the inflammatory process.[5,11,13,16]

Overexpression of proinflammatory innate immune products IL-1β, TNF-α, IL-6, and IL-13 is well documented

Received for publication May 2, 2008; Accepted May 9, 2008.

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DOI 10.1002/ibd.20527

Published online 14 July 2008 in Wiley InterScience (www.interscience.wiley.com).

in UC.[17,20] Tissue levels of these proinflammatory cytokines correlate with disease activity,[21–23] and their selective inhibition has been shown to attenuate the onset of experimental UC.[14,20] Increased expression of IL-4, IL-5, IL-8, and IL-18 also appears to accompany UC.[17,24,25] In normal hosts, tolerance to immune response is mediated by regulatory T cells, B lymphocytes, natural killer T cells, and dendritic cells that secrete transforming growth factor (TGF)-β, IL-10, interferon (IFN)-α/β, and prostaglandin J2.[17] However, in recent years UC has been increasingly associated with an imbalance of a newly identified T-cell subset, Th17 CD4+ T cells, which differentiate primarily upon stimulation of naïve CD4+ T cells in the presence of IL-23, IL-1β, and IL-6 via activation of STAT3 and ROR-γt.[26–29] Th17 T cells are thought to promote responses against extracellular pathogens such as bacteria and fungi[30] and secrete IL-17A/F, IL-21, IL-22, defensins, and TNF-α.[31,32] Additionally, aberrant IFN-γ expression has been associated with UC, which is produced predominantly by natural killer (NK), natural killer T (NKT) cells as part of the innate immune response, and by Th1 CD4 and CD8 cytotoxic T lymphocyte (CTL) effector T cells once antigen-specific immunity develops.[33] IFN-γ was shown to be involved in perpetuation of experimental colitis, possibly through induction of excessive nitric oxide (NO) activity.[34] Colonic bacteria produce NO, which has been implicated in the pathophysiology of intestinal inflammation.

Conventional therapeutic strategies for UC generally require a trial-and-error approach to tailor a treatment that will be both effective and tolerable for any given patient.[35] The most common drug arsenal used for UC includes aminosalicylates (including sulfasalazine), corticosteroids, immunomodulators (cyclosporine [CSA], azathioprine, 6-mercaptopurine), and most recently biologics. The first line of therapeutics generally consists of aminosalicylates alone or in combination with corticosteroids during acute episodes and aminosalicylates and/or immunomodulators to maintain remission.[4] Aminosalicylates exert antiinflammatory effects through inhibition of IL-1, IL-2, and NF-κB, while corticosteroids exert potent immunosuppressive effects, including suppression of the arachidonic acid cascade, IFN-γ, and IL-1, -2, -4, -5, -6, and -8.[4] CSA, an immunosuppressant drug, has been used as a salvage therapy for patients with severe refractory UC, also with long-term immunomodulation with thiopurines, particularly azathioprine or 6-mercaptopurin.[36] However, these latter drugs have a slow onset of action, are less than 50% effective long-term, and are associated with important side effects in some patients.[37] Alternative therapeutic approaches target gastrointestinal (GI) motility and increased fluid and micronutrient reabsorption such as loperamide, codeine sulfate, or tincture of opium.[35] Anti-p40 therapy, initially thought to inhibit IL-12 as a Th1 promoter, has proven beneficial, although the recent understanding of UC biology suggests that this effect was secondary to the inhibition of IL-23 (which shares p40 with IL-12) that blocked recruitment/differentiation of Th17 T cells to the GI tract,[28,38] suggesting that restoration of GI immunological imbalance may a promising strategy to ward off clinical symptoms of UC while minimizing side effects to the patient. Overall, there is a continuing need for additional effective, safe, tolerable treatments for UC.

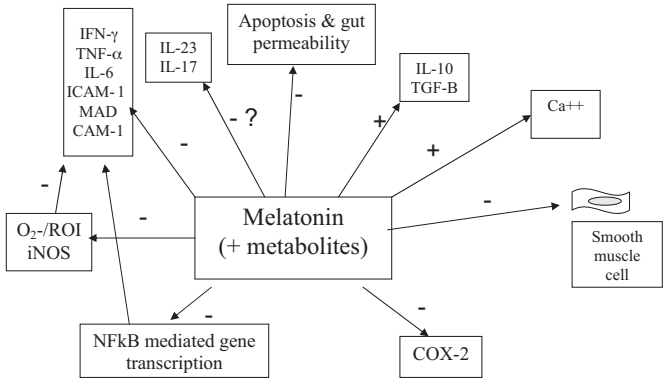

FIGURE 1. Pathways regulated by melatonin.

MELATONIN AND ULCERATIVE COLITIS

Melatonin and GI Tract Physiology and Health

Melatonin was first described as a secretion from the pineal gland with multiple neurohormonal functions, including regulation of the circadian rhythm, reproductive physiology, and body temperature, but has since also been found to inhibit the Cox-2 and NF-κB pathways and several aging processes (reviewed by Reiter et al[39]). The multifactorial role of this hormone, however, has only relatively recently been appreciated (Fig. 1) as it circulates unimpeded across anatomical barriers, the blood–brain barrier included, and exhibits both receptor-dependent and receptor-independent effects. Furthermore, melatonin exhibits a high degree of conservation across the evolutionary ladder, pointing to a critical function in various forms of life, even in organisms devoid of a pineal gland. In fact, the analysis of extrapineal sources of melatonin have highlighted the GI tract as a major source of this factor, with concentrations of melatonin as much as 100 times that found in blood and 400 times that found in the pineal gland.[40] GI melatonin comes from both pineal melatonin and de novo synthesis in the GI tract and may have a direct effect on many GI tissues, serving as an endocrine, paracrine, or autocrine hormone, influencing the regeneration and function of epithelium, modulating the immune milieu in the gut, and reducing the tone of GI muscles by targeting smooth muscle cells.[40] Melatonin may also influence the GI tract indirectly, through the central nervous system and the mucosa, by a receptor-independent scavenging of free radicals leading to reduction of inflammation, reduction of secretion of hydrochloric acid, stimulation of the immune system,

fostering tissue repair and epithelial regeneration, and increasing microcirculation.[4,40]

Human intestinal motility follows a circadian rhythm with reduced nocturnal activity.[41–43] Abnormalities in colonic motor function in patients with UC have been well documented.[44–46] Melatonin appears to be involved in the regulation of GI motility, exerting both excitatory and inhibitory effects on the smooth musculature of the gut.[40,47] The precise mechanism through which melatonin regulates GI motility is not clear, although some studies suggest that this may be related to blockade of nicotonic channels by melatonin[48] and/or the interaction between melatonin and Ca2+ activated K+ channels.[49] Melatonin may also function as a physiological antagonist of serotonin.[40,50] In a recent rodent model, melatonin administration was shown to reverse lipopolysaccharide-induced GI motility disturbances through the inhibition of oxidative stress.[51] The net motor regulation by melatonin is, therefore, likely multifactorial.

In addition, several lines of in vitro studies,[52,53] as well as animal studies,[54–57] have reported that melatonin regulates the extensive gut immune system and has important general antiinflammatory and immunomodulatory effects. Given its presence in GI tissue and its suggested importance in GI tract physiology, it is reasonable to hypothesize that melatonin could influence inflammation-related GI disorders, including UC. In various animal experiments, melatonin administration was (among other immunomodulatory effects) shown to increase IL-10 production and inhibit production of IFN-γ, TNF-α, IL-6, and NO,[51,54,58] suggesting that melatonin may exert benefits in UC by reducing or controlling inflammation. Melatonin administration has also inhibited the TNF-α-induced mucosal addressin cell adhesion molecule (MAdCAM)-1 in vitro,[59] and intercellular adhesion molecule (ICAM)-1 in vivo,[60] limiting the influx of activated $\alpha 4\beta 7$+ and LFA-1+ leukocytes to the mucosal environment. During inflammation, the mucosal microvasculature controls the selection and magnitude of influx of T-cell subsets into the gut through cell adhesion molecules expression and chemokine secretion, which further amplify the communication with other leukocytes and cells.[17,61] In animal experiments neutralization of MAdCAM-1 and ICAM-1 led to attenuation of mucosal damage in colitis.[13,62,63]

Animal Experiments of Melatonin Administration and Colitis

At least 13 experiments in rodents have shown that melatonin administration reduces the severity of colitis (Table 1).[64–76] These effects were attributed to a variety of mechanisms, including inhibition of NO production,[67,71] inhibition of COX-2 expression,[67] inhibition of NF-kappa activation,[67,68] reduction of colon immunological injury through regulation of macrophage activity,[72] reduction of proinflammatory cytokines,[70,72,77] reduction of bacterial translocation,[64] reduction of matrix metalloproteinase-2 and -9 activity,[77] and modulation of signal transduction pathways and apoptosis.[64,70] Only 1[69] of these rat studies showed a worsening of colitis with the highest doses of melatonin used long-term, even though short-term benefits were shown with all examined doses in that study. However, those results were not replicated by any of the other studies that examined similarly induced colitis using the same (or higher) dose and duration of treatment. Future studies may delineate the various levels of the inflammatory pathways that may be influenced directly and indirectly by the administration of melatonin.

Human Studies

In addition to the basic science data and experimental studies, results from human studies provide several lines of evidence that suggest that melatonin supplementation could have an ameliorative effect on UC.

Studies in humans have indicated that melatonin supplementation can help combat inflammation and oxidative stress, 2 of the pathophysiologic factors involved in UC. Indeed, trials have shown that melatonin can reduce inflammatory and/or oxidative stress markers in a range of human subjects including infants with respiratory disease,[78] adults with sporadic amyotrophic lateral sclerosis (ALS),[79] and healthy adults.[80,81] Of note, 2 of these studies[78,80] found that melatonin supplementation reduced TNF-α levels, a factor that is believed to be especially important in UC.[82]

There have been several anecdotal findings of melatonin supplementation alleviating UC, including a published case report of patient with UC who intermittently took melatonin to ameliorate jet lag, each time resulting in the disappearance of his UC symptoms.[83] Investigators in India have cited their own (unpublished) data showing "some improvement in symptoms in patients with severe, refractory colitis by melatonin administration."[84] Melatonin used to treat children with sleep disorders appeared to significantly improve their existing GI conditions, including UC.[85,86] In contrast, there is a recent case report of a man whose UC symptoms reappeared 2 months after starting to take daily melatonin for sleep promotion that subsided shortly after discontinuing melatonin.[87] The reasons for the conflicting observations are unclear. Case reports such as these can be valuable, for example, in generating hypotheses regarding poorly understood diseases,[88] although their limitations in etiological research have been widely recognized.

Observational epidemiological studies have been scarce. A large follow-up study in Germany found increased prevalence of UC and total IBD among men and women who engaged in extended or irregular shift work,[89] which itself has been associated with lower endogenous melatonin levels.[90] Shift work has also been linked with increased colorectal cancer risk,[91] possibly through melatonin-mediated mecha-

TABLE 1. Animal Experiments of Melatonin and Ulcerative Colitis

Study, Year	Animal/Qualification	Melatonin Dose	Duration of Melatonin Treatment	Main Findings
Pentney, 1995[75]	Mice with DSS-induced colitis	150 mcg/kg/d (intraperitoneal)	7 weeks	Melatonin reduced severity of colitis and focal lesions. Rectal bleeding and occult blood eliminated.
Cuzzocrea, 2001[66]	Rats with DNBS-induced colitis	15 mg/kg/d, i.p.	1 week	Melatonin reduced bloody diarrhea, weight loss, colonic MPO activity, MDA levels, nitrotyrosine and PARS immunoreactivity in the colon, up-regulation of ICAM-1, expression of P-selectin, COX-2 and INOS staining, and ameliorated disruption of the colonic architecture.
Mei, 2002[72]	Rats with DNBS-induced colitis	2.5, 5, 10 mg/kg/d (intrarectal)	3 weeks	Melatonin reduced colon mucosa damage and occult blood similar to 5-ASA, suppressed levels of IL-1, TNF-α, NO.
Dong, 2003[67]	Sprague-Dawley (S-D) Rats with TNBS-induced colitis	2.5, 5, 10 mg/kg/d (intrarectal)	21 or 27 days	Melatonin reduced colonic injury induced by acetic acid or TNBS, possibly by local inhibition of iNOS and/or COX-2.
Mei, 2005[71]	Rats with acetic acid- or TNBS-induced colitis	2.5, 5, 10 mg/kg/d (intrarectal)	3 weeks	Melatonin reduced mucosal damage index, histology score, MDA, and NO in vivo, reduced oxidative injury in vitro.
Li, 2005[68]	Rats with TNBS-induced colitis	2.5, 5, 10 mg/kg/d (intrarectal)	4 weeks	Melatonin reduced colonic inflammatory injury similar to 5-ASA, in part through NF-kappaB inhibition.
Cevik, 2005[65]	Rats with acetic acid-induced colitis	Exposure to complete darkness vs. normal light cycle	15 days prior to colitis induction	Colonic injury was reduced by exposure to complete darkness (increased melatonin levels) prior to colitis induction. Antioxidant mechanisms were suggested.
Marquez, 2006[69]	Wistar Rats with TNBS-induced colitis	0.5, 1, 2 mg/kg, i.p.	1 – 3 weeks	Melatonin reduced the severity of colitis short term, but long term administration (2 mg / kg) worsened colitis with increase in MPO activity and TNF-α production.
Necefli, 2006[73]	Male Wistar rats	10 mg / kg, i.p.	15 days	Melatonin reduced mucosal damage due to anti-inflammatory and anti-apoptotic effects.
Mazzon, 2006[70]	Rats with DNBS-induced colitis	15 mg/kg/d i.p.	4 days	Melatonin ameliorated colonic disrupted architecture and reduced TNF-α, NF-kappaB, phosphorylation of c-Jun, Fas ligand, and bax expression, and the loss of Bcl-2.
Nosal'ova, 2007[74]	Rats with acetic acid-induced colitis	5, 10 mg/kg	48 hours	Melatonin reduced colonic inflammation, possibly by preserving GSH reserve, preventing lysosomal enzyme disruption, and inhibiting MPO activity.
Akcan, 2008[64]	Wistar rats with TNBS-induced colitis	10 mg/kg	10 days	Melatonin reduced incidence of bacterial translocation to the liver, spleen, mesenteric lymph nodes, portal and systemic blood and significantly decreased caspase-3 activity in colonic tissues.
Esposito, 2008[76]	S-D rats with DNBS-included colitis	15 mg/kg/d i.p	4 days	Melatonin prevented colon injury and lipid peroxidation, and reduced MMP-9, MMP-2, and TNF-α expression.

nisms similar to those that may underlie associations with UC.[92] A recent case–control study in Poland showed that levels of 6-hydroxymelatonin sulfate, a melatonin metabolite, in urine samples from UC patients were higher than those from healthy controls, yet in UC patients they were inversely associated with severity of UC.[93] Although these 2 findings may appear contradictory, the investigators concluded that "melatonin seems to be a part of anti-inflammatory response and its high level may appease the course of UC." More consistent with the latter finding, Lu et al[94] showed endoge-

TABLE 2. Clinical Trials of Melatonin and Irritable Bowel Syndrome (IBS) in Humans

Study, Year	Population/Study Design	Melatonin Dose	Duration of Melatonin Treatment	Main Findings
Song, 2005[96]	16 male and 24 female IBS patients (aged 20-64 years) with sleep disturbance randomized to take melatonin or placebo	3 mg/d or placebo at bedtime	2 weeks	Melatonin significantly decreased mean abdominal pain score (2.35 vs. 0.70; P<0.01), not through sleep parameters. Bloating, stool type, and stool frequency, were not different between the two groups.
Lu, 2005[94]	17 Female IBS patients(aged 24-66 years) randomized to take melatonin or placebo, and 18 control subjects to compare baseline saliva melatonin levels	3 mg/d or placebo	8 weeks	Melatonin improved mean IBS scores (3.9 + 2.6) compared with placebo (1.3 + 4.0; P=0.07). Percentage of subjects achieving mild-to-excellent improvement in IBS symptoms was also greater in the melatonin-treated vs. placebo arm (88% vs. 47%; P=0.04). The melatonin arm showed significant improvement in abdominal pain scores, abdominal distension scores, and abnormal sensation of defecation scores, not through sleep patterns IBS patients had lower endogenous melatonin levels than controls.
Saha, 2007[95]	12 male and 6 female IBS patients (aged 18-65 years) randomized to take melatonin or placebo	3 mg/d or placebo at bedtime	8 weeks	Melatonin significantly improved overall IBS score (45% vs. 16.66%; P<0.05), posttreatment overall extracolonic IBS score (49.16% vs. 13.88%; P<0.05), and Quality of Life score (43.63% vs. 14.64%; P<0.05).

nous saliva melatonin levels of irritable bowel syndrome (IBS) patients were significantly lower than those of healthy controls.

Finally, 3 clinical trials of melatonin supplementation (3 mg/d) versus placebo among patients with IBS all showed dramatic, statistically significant reductions in symptoms in the treatment group,[94–96] with treatment times ranging from 2–8 weeks (Table 2). These results may have implications for UC because there is a growing appreciation that IBS and UC, although distinct conditions share some clinical manifestations, and that both diseases may be driven, at least in part, by proinflammatory/prooxidative stress mechanisms.[97] To date, there has been no formal clinical trial of melatonin therapy for UC in humans, and there have been few mechanistic investigations into the potential mode of action of this compound on the pathomechanisms identified thus far. Given the very low toxicity documented for even high levels of melatonin in animals and human subjects, this relatively inexpensive treatment may represent an attractive alternative for patient suffering from mild to mid-grade UC given the low incidence of side effects. However, the success of such therapy may be secondary to individual sensitivity to the action of melatonin, hence the need for adequate attention to timing and dosages, which may be based, at least initially, on the results of studies of melatonin in support of other conditions, such as sleep disorders.[39]

CONCLUSIONS

UC is a common GI disorder in adults, especially in Western countries, one that can have debilitating symptoms and severe health consequences. Current treatments are not always effective, and often have serious side effects. Therefore, there is considerable interest in finding alternative, less toxic treatments for this disease. Physiologic data suggest that melatonin plays an important regulatory role in GI tract physiology and health, and data from in vitro studies, animal experiments, and limited studies in humans suggest that supplemental melatonin may have an ameliorative effect on colitis. However, the evidence is lacking in several ways, including the dearth of randomized clinical trials in humans, even though such trials have been deemed important.[4,40] Furthermore, identifying sets of objective clinical parameters in addition to patient reports will be important in providing a platform with which melatonin therapy may be optimized and/or combined with additional forms of therapy in a way that is more compatible with the quality of life of individual patients.

In vitro studies and analyses of tissue samples obtained from animal experiments using melatonin supplementation on UC have revealed several potential mechanisms of action, including those related to melatonin's inhibition and/or suppression of specific inflammation-related cytokines and cell adhesion molecules. These have been deemed important in

the molecular basis of UC pathology in humans as well. However, it is important to view findings from animal experiments in light of the uncertain relationship between chemically induced UC in rodents and UC in humans. Recent findings in both animal models and human patients provide a refined framework of immunopathomechanisms potentially at the origin of UC; hence, their evaluation should be included within the context of clinical trials of melatonin supplementation.

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