World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i34.3888

World J Gastroenterol 2011 September 14; 17(34): 3888-3898
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Distribution, function and physiological role of melatonin in the lower gut

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Received: January 11, 2011 Revised: March 18, 2011
Accepted: March 25, 2011
Published online: September 14, 2011

Abstract

Melatonin is a hormone with endocrine, paracrine and autocrine actions. It is involved in the regulation of multiple functions, including the control of the gastrointestinal (GI) system under physiological and pathophysiological conditions. Since the gut contains at least 400 times more melatonin than the pineal gland, a review of the functional importance of melatonin in the gut seems useful, especially in the context of recent clinical trials. Melatonin exerts its physiological effects through specific membrane receptors, named melatonin-1 receptor (MT1), MT2 and MT3. These receptors can be found in the gut and their involvement in the regulation of GI motility, inflammation and pain has been reported in numerous basic and clinical studies. Stable levels of melatonin in the lower gut that are unchanged following a pinealectomy suggest local synthesis and, furthermore, implicate physiological importance of endogenous melatonin in the GI tract. Presently, only a small number of human studies report possible beneficial and also possible harmful effects of melatonin in case reports and clinical trials. These human studies include patients with lower GI diseases, especially patients with irritable bowel syndrome, inflammatory bowel disease and colorectal cancer. In this review, we summarize the presently available information on melatonin effects in the lower gut and discuss available *in vitro* and *in vivo* data. We furthermore aim to evaluate whether melatonin may be useful in future treatment of symptoms or diseases involving the lower gut.

Key words: Melatonin; Ileum; Colon; Receptor; Motility; Inflammatory bowel disease; Clinical trial

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Chen CQ, Fichna J, Bashashati M, Li YY, Storr M. Distribution, function and physiological role of melatonin in the lower gut. *World J Gastroenterol* 2011; 17(34): 3888-3898 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i34/3888.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i34.3888

INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine), discovered in 1917, is found in humans, animals, plants and microbes. It is a lipophilic compound diffusing rapidly through biological membranes and is involved in many regulatory processes, such as biological rhythms, intestinal reflexes, protection against inflammation, metabolism and repro-

duction. Additionally, melatonin may act as a mediator of inter-organ communication, e.g., between gut and liver[1].

In animals, melatonin was first reported in the bovine pineal gland in the year 1958, and in the gut it was first identified in the human appendix in 1974[2]. Melatonin is synthesized in the pineal gland and secreted in a circadian pattern, with highest amounts released during nighttime[3]. The light/dark information regulating the secretion of melatonin by the pineal gland is received in the supra-chiasmatic nuclei *via* retinal photosensitive ganglionic cells. Melatonin released into the bloodstream acts as an endocrine hormone and controls biological functions with circadian rhythms, e.g., the sleep-wake cycle. Melatonin is also involved in the regulation of food intake and digestion[4].

Following the detection of the melatonin-synthesizing enzymes N-acetytransferase and hydroxyindole-O-methyltransferase in the gut[5], the possibility of additional extra-pineal melatonin synthesis was considered[6]. Melatonin produced in the gut is believed to act as a paracrine hormone which can be secreted in both a continuous or a cyclic fashion. Melatonin is also synthesized by a variety of other extra-pineal cells, such as bone marrow cells, lymphocytes, mast cells and epithelial cells, and it is unclear to what extent melatonin from these sources contributes to gut melatonin levels. Release of melatonin from all these extra-pineal sources seems to be independent of the photoperiod[7-9].

Melatonin has been studied in different areas of medicine in numerous clinical trials. In the lower gut, the roles of melatonin are complex and largely uncharacterized. This review will focus on the gastrointestinal (GI) localization of melatonin, the role of melatonin in the lower gut and the mechanisms involved. Animal and benchside research, as well as translational human research, will be discussed with a special emphasis on summarizing all available human data related to the lower gut.

MELATONIN PRESENCE IN THE LOWER GUT

Bubenik *et al*[10] were the first to report the presence of melatonin in the mucosa of the gut. This observation was later confirmed by studies using immunohistochemistry and radioimmunoassay techniques[11]. In the mammalian gut, melatonin exhibits striking differences in regional distribution, with the highest levels in the rectum and the colon and the lowest levels in the jejunum and the ileum. These regional differences in tissue distribution were confirmed in other species including rabbit, mouse and human[12]. Moreover, specific antibodies against melatonin in rat identified melatonin-like immunoreactivity in all parts of the gut, and after administering exogenous melatonin the most pronounced accumulation of melatonin was seen in the colon and the rectum[13]. Furthermore, melatonin was detected in luminal fluids of the gut. This melatonin may originate from food, mucosal sources, or organisms populating the gut. Finally, luminal melatonin may also be of biliary origin, but at the present time its sources have not been elucidated.

Melatonin is synthesized in the enterochromaffin (EC) cells throughout the gut[14,15], and the EC cells have been reported to be the major source of L-tryptophan-induced increase of circulating melatonin. Interestingly, the distribution of melatonin is comparable to the density of EC cells in the gut. Oral administration of L-tryptophan caused a rapid and dose-dependent elevation of circulating melatonin in rats or chickens[16]. L-tryptophan-induced melatonin synthesis was greater following oral than following intraperitoneal administration. This indicates that L-tryptophan is a crucial precursor in gut melatonin synthesis.

Some melatonin detected in the gut is of pineal origin through accumulation from circulating sources, and the digestive tract, especially in the lower gut, might act as a store for pineal-derived melatonin particularly at nighttime[17]. Melatonin levels in the gut are independent of pineal production, since in rats pinealectomy had no influence on gut melatonin concentrations[18]. Interestingly, at any time of the day or night, the gut contains at least 400 times more melatonin than the pineal gland, once again emphasizing the functional importance of melatonin in the gut. No photoperiodic cyclical secretion of melatonin was observed in the gut, which is in contrast to the typical secretion pattern for melatonin from the pineal gland. In diabetic rats, lower melatonin levels were observed in the pancreas, the kidneys and the duodenum, but no change of melatonin level was detected in the colon, when compared to non-diabetic control rats[19]. The relevance of these observations has yet to be determined.

Melatonin concentrations in the gut vary depending on age. In the postnatal rat, GI melatonin levels peaked at birth and then declined to stable levels at the age of 21 d[13]. This decline in melatonin concentrations was more pronounced in the jejunum, ileum and colon compared to the stomach[20]. However, later in life the levels of melatonin increase; it has been shown that melatonin concentration in the mucosa of the ileum and distal colon is 126% higher in older mice (22-24 mo) compared to younger mice (2-5 mo)[21]. Remarkably, the same study provided evidence that most of the daytime levels of melatonin in the blood are of GI origin. It was also shown in pigs that the serum melatonin levels correlate well with the levels of melatonin in the lower gut[22]. Food restriction increases melatonin concentrations in the gut and in the brain in mice[23]. These distinct changes in melatonin levels suggest that there may be a physiological role for melatonin in the regulation of digestion and in the control of food intake. On the contrary, the melatonin levels in the lower gut may be influenced by luminal contents and may thus depend on the movement of digesta, but this notion remains speculative at the present stage.

MELATONIN RECEPTORS ARE LOCALIZED IN THE ILEUM AND THE COLON

Melatonin exerts some of its physiological effects through

activation of specific membrane receptors. According to their pharmacological properties, these receptors have been classified as Mel1A, Mel1B and Mel1C[24,25]. Two of these, Mel1A and Mel1B, were recently renamed melatonin-1 receptor (MT1) and MT2 receptors. Both MT1 and MT2 receptors are members of the G-protein coupled receptor family and share a common seven transmembrane structure. MT1 and MT2 show high homology at the amino acid level, with a 55% overall homology and a 70% homology within the transmembrane domains. They also share some specific short amino acid sequences, suggesting that they represent a specific subfamily[26,27]. However, MT1 and MT2 receptors activate very distinct intracellular signaling pathways[28,29]. It has been shown that the MT1 melatonin receptor is coupled to G proteins that mediate adenylate cyclase inhibition and phospholipase C beta activation. The MT2 receptor couples to a number of signal transduction pathways, including phosphoinositol production, inhibition of adenylate cyclase and the inhibition of soluble guanylate cyclase pathway[30-32]. Luzindole acts as an antagonist at both receptors and is used in numerous studies[33-35]. 4-P-PDOT is a selective antagonist at MT2[36]. For the MT1 receptor no highly selective antagonist has been reported yet.

The third melatonin binding site MT3 (formerly Mel1C) is an enzyme named quinone reductase 2 (QR2). MT3 can be blocked by prazosin. Activation of MT3/QR2 by melatonin may explain the protective effect of melatonin against oxidative stress in different animal models, since MT3/QR2 has potent antioxidant properties.

The melatonin receptors display regional tissue and cell specific variations, reflecting the overall complexity of melatonin signaling[37]. Functional assays, as well as receptor binding studies, have demonstrated the presence of high affinity binding sites for melatonin on cell membranes, for example in the hypothalamus, medulla oblongata, hippocampus, cerebellum, parietal cortex and striatum of rats. Interestingly, the density of these binding sites varies depending on the time of the day and physiological conditions, including age[38,39].

Furthermore, there exists a group of nuclear melatonin receptors. These nuclear receptors for melatonin belong to the retinoid Z receptor (RZR) or retinoid orphan receptor (ROR) subfamilies, which include three subtypes (α, β, γ), encoded by three different genes[40,41]. An interaction between membrane and nuclear melatonin receptors was suggested by the observation that the expression of ROR/RZR mRNA is decreased in blood mononuclear cells with reduced MT1 receptor expression. Finally, melatonin can directly interact with intracellular proteins such as calmodulin, calreticulin or tubulin, extending the list of potential sites of binding and action for melatonin[42-44].

All three MT receptors can be found in the gut and the data on localization are summarized in Table 1. The subcellular distribution of melatonin binding is highest in the nuclear fraction, followed by the microsomal and the mitochondrial fractions, and is lowest in the cytosolic fraction.

Melatonin MT1 receptor mRNA has been detected in rat small and large intestine. The highest MT1 mRNA expression was found in the rat duodenum, with lower expression in the jejunum and ileum. No circadian changes were found in MT1 mRNA expression in gut tissues. In the duck gut, there was found to be a significant variation in the densities of 2-iodo (^{125}I)-melatonin binding sites in different regions of the gut, with the following descending order of density: ileum, jejunum > duodenum, colon > cecum > esophagus[45]. Short-term fasting increased the expression of MT1 in the subepithelial layer of the rat small and large intestine, but no changes in MT1 expression were detected in other gut layers. During long-term fasting this increase in MT1 expression persisted only in distal colon, while in the remainder of the colon and in the small intestine MT1 expression returned to normal levels[46-48].

Table 1 Localization of melatonin membrane receptors in the ileum and the colon of rodents

	MT1	MT2	MT3
Ileum	+	+	+
Colon	+	+	+
Mucosa	+	No data	No data
Muscularis mucosae		+ (i); + (c)	
Submucosa		+ (i); + (c)	
Muscularis propria		+ (c)	

MT: Melatonin receptor; i: Ileum; c: Colon.

A study using tissues from rat pancreas, stomach, duodenum and colon found the highest levels of MT2 in the colon by using western blot analysis[49]. In the same study, the most intense MT2 immunoreactivity was observed in the muscularis mucosae and in the circular and longitudinal muscle layers of rat gut. Detection of MT2 receptors in the gut muscle layers suggests an involvement of MT2 in the regulation of intestinal motility. Comparable to MT1, the expression of MT2 receptors does not vary with food intake.

Pharmacological studies suggested the presence of the melatonin MT3 receptor in guinea pig colon[50] and later MT3 was found in monkey (*Macaca fascicularicus)* gut[51]. However, presently it is unclear in which gut layer MT3 is expressed.

In addition, in blood vessels of both rodent and human colon, a high density of melatonin-binding sites was reported. *In vitro* preparations of arterial smooth muscle of the porcine colon relax in response to melatonin and melatonin receptor agonists, although these effects were seen at rather high concentrations of melatonin[52]. Based on *in vitro* experiments on rat arteries, it has been suggested that a vasoconstrictive effect of melatonin is mediated *via* MT1 and a vasodilatatory effect is mediated *via* MT2 receptors[53]. The effects of melatonin in the gut may be dose-dependent and reflect actual MT1/MT2 ratio in muscle layers of gut segments.

Whereas data are available for the localization and expression of MT receptors in the gut, the presence of nuclear melatonin binding sites remains unresolved. One

study has suggested that melatonin nuclear receptors are present in murine colon cancer cells, but the relevance of this observation and localization remains unclear[54].

ACTIONS OF MELATONIN IN THE ILEUM AND THE COLON

In contrast to the central nervous system, the function of melatonin in the gut is less clear. In the gut it seems that melatonin plays significant roles in regulating intestinal motility, the immune system, GI secretion, and the release of peptides involved in energy balance such as peptide YY[55]. Melatonin was also shown to protect the colon in different pathophysiological conditions; frequently these protective effects involve activation of antioxidative mechanisms or the regulation of blood vessel tone and thus modification of perfusion[56,57]. Another effect of melatonin is the alteration of gut flora and potential anti-microbiotic actions; melatonin was shown to influence *E. coli* O157:H7 growth *in vitro* and *in vivo* in infected wethers[58].

Motility

Melatonin is known to be involved in the regulation of GI motility. Melatonin is produced in EC cells of the GI tract and has high lipophilicity, and therefore may diffuse into deeper layers through mucosa and submucosa, to finally act in the muscularis mucosae or the myenteric plexus. In these actions, muscular and neuronal sites are involved. Contractile and relaxant effects of melatonin in the GI tract have been reported in numerous species[59-61]. The involved sites of action and the mechanism of melatonin action in the GI tract are not poorly characterized. Involvement of melatonin receptors and/or ion channels located on GI smooth muscle cells and/or neurons have been suggested and details are discussed below.

Melatonin alters GI motility by activating melatonin receptors. The most likely sites of melatonin action in the GI smooth muscle cells are the membrane-bound melatonin receptors and there is strong evidence that MT2 receptors are involved. *In vivo* animal studies showed that melatonin exerted both excitatory and inhibitory effects on the gut depending on the dose of melatonin. Small doses of melatonin accelerated the intestinal transit in rats, while high doses reversed this effect. These effects were blocked by luzindole, suggesting the involvement of intestinal melatonin receptors[62].

Early *in vitro* research showed that melatonin reduces the force of spontaneous contractions of ileum and colon segments of rat intestine, while the frequency of intestinal contractions remained unchanged[61]. In the GI tract, the cyclic generation of electrical currents is one fundamental mechanism of coordinated smooth muscle contraction. Slow waves and spiking activity are organized in myoelectric migrating complexes (MMC). Depending on the report, endogenous and exogenous melatonin inhibits pre- and postprandial irregular spiking activity of intestinal motility. Furthermore, pinealectomy suppressed the regular phase of MMCs, and administration of exogenous melatonin could restore a regular phase MMC activity in rat ileum[63]. These changes may depend on the action of melatonin on the GI neurons. In one study focusing on gastric emptying, melatonin partly inhibited gastric motility by activating sympathetic neurons. In the stomach, melatonin also reduces nitrergic myenteric innervation[64]. In electrophysiological experiments it was shown that the nitrergic component of the smooth muscle inhibitory junction potential was reduced by melatonin and this may be a consequence of direct inhibition of nitric oxide synthase (NOS) activity by melatonin at enteric synapses. Other studies suggest that the effect of melatonin may be related to the blockade of nicotinic channels by melatonin, or due to an interaction between melatonin and Ca^{2+}-activated K^+ channels[65]. Furthermore, it was demonstrated that the inhibitory effect of melatonin is apamin-sensitive and thus involves Ca^{2+}-activated K^+ channels[66]. One study also showed that the melatoninergic attenuation of acetylcholine-induced contractions of intestinal strips from goldfish is dependent on extracellular calcium[67].

Moreover, a beneficial effect of melatonin in reversing lipopolysaccaride-induced motility disturbances, which involves a reduction in lipid peroxidation and an increase of mitogen-activated protein kinase activation, nuclear factor kappaB (NF-κB) activation, inducible NOS (iNOS; NOS-2) expression and finally nitrite production[68], was reported. Additionally, melatonin was shown to modulate the cholecystokinin action on ileal motility and to reduce the duration of cholecystokinin stimulatory effects on GI smooth muscle in rats[69].

Other possible sites of melatonin action are 5-HT receptors. One study suggested that high doses of melatonin in the GI tract interact with cholecystokinin-2 and 5-HT3 receptors on the vagal afferent fibers, and thus induce vago-vagal inhibitory reflexes[70]. In some reports, the relaxant effect of melatonin through 5-HT receptor antagonism was proven[71], but other pathways may also be involved. Recently, it was demonstrated that melatonin can inhibit the activity of the serotonin transporter, which controls the reuptake of 5-HT in intestinal epithelial cells and inhibits NK_2 receptor-triggered 5-HT release from guinea pig colonic mucosa by acting at a MT_3 melatonin receptor located directly on the mucosal layer[72]. These actions may at the same time affect gut secretion.

Secretion

Melatonin is involved in the regulation of intestinal ion transport. Exogenous melatonin reduced diarrhea in rats with colitis, but the involved mechanisms have not been fully elucidated[73]. In the colon, melatonin is thought to play a role in regulating Cl^- secretion[74]. Melatonin can affect the expression of COX-2 and iNOS and melatonin modulates secretion elicited by prostaglandin E_2 and sodium nitroprusside in rat distal colon. Some of these secretory effects seem to be localized in the colonic epithelium and involve cAMP pathways, while others involve the enteric neuronal system[75]. According to these studies,

melatonin is a physiological modulator of ion transportation in the lower gut and many mechanisms are involved.

Immune system

Melatonin has numerous effects on the immune system. It increases natural killer cell activity and Th2 cell-mediated immune responses[76,77]. Melatonin was reported to regulate gene expression of several cytokines including IL-2, IL-2R and IFN-γ released by human CD4 T cells[78,79]. The effects on other functions of the immune system, such as lymphoproliferation and cytokine production by human lymphocytes, have also been studied. Melatonin protects human and murine $CD4^+$ T cells from apoptosis by inhibiting CD95 ligand mRNA and protein up-regulation in response to TCR/CD3 stimulation[80]. Additionally, the melatonin/IL-2 relationship may be particularly relevant for immune tolerance. Melatonin can affect T-cell tolerance *via* IL-2[81]. At the same time, melatonin acts as an immunomodulator and these effects are mediated by melatonin receptors located on immunocompetent cells[82]. Melatonin synthesized in human lymphocytes is involved in the physiological regulation of IL-2/IL-2R expression through mechanisms comprising both membrane and nuclear melatonin receptors[83].

The majority of melatonin effects described for lymphocytes seem to be mediated through MT1 receptors[84]. However, some evidence shows that melatonin-induced enhancement of immune function is also mediated *via* MT2 receptors[85]. Antagonists at the MT2 receptor or the nuclear RZR/ROR were found to reduce human lymphocyte IL-2 production, proving the involvement of these binding sites in IL-2 production[86].

Experimental inflammation

By preserving the mucosal cell integrity and inhibiting the accumulation of neutrophils, melatonin exerts protective effects against inflammation in the gut[87]. Melatonin was shown to reduce the severity of intestinal inflammatory pathologies such as colitis in animal models[88]. Pentney *et al*[89] reported that daily melatonin administration reduced the severity of dextran sodium sulphate (DSS)-induced colitis in mice. In these experiments, serum melatonin levels were more than 10 times higher in mice that received DSS, as compared to controls. It is presently unclear what causes the significant improvement of inflammation in melatonin-treated mice, as no receptor antagonists were employed in this study and no downstream mechanisms were investigated. Melatonin has been reported to reduce the severity of experimental colitis in mice and rats and though *in vitro* and *in vivo* studies suggest numerous pathways involved, the exact mechanism of action remains unclear[90]. In experimental colitis in rats, melatonin reduced colon injury by influencing numerous events including the enzyme activities of matrix metalloproteinase-9 (MMP-9), MMP-2 and caspase-3, by suppressing the activities of cyclooxygenase-2 (COX-2) and iNOS, inhibiting the expression of NF-κB and acting as a radical scavenger[91-95]. Moreover, the regulation of macrophage activity[96] and the reduction of bacterial translocation in trinitrobenzene sulfonic acid (TNBS)-induced colitis have been reported[97]. Melatonin treatment also causes a substantial reduction of *FasL* gene activation, which is known to induce a pro-inflammatory response characterized by a release of IL-1b, macrophage inflammatory protein-1a (MIP-1a), MIP-1b and MIP-2. Blocking the action of these cytokines has been shown to delay the onset of experimental colitis, to suppress inflammation and to ameliorate colonic damage[98]. But melatonin does not exert unanimously protective effects. Marquez *et al*[99] reported that acutely administered melatonin is protective against TNBS-induced colitis in rats, whereas chronic melatonin treatment exaggerates colitis. Future studies are needed to clarify the full extent of melatonin protection against colitis and to characterize the involved mechanisms.

ROLE OF MELATONIN IN DISEASES INVOLVING THE ILEUM AND THE COLON

Irritable bowel syndrome

Irritable bowel syndrome (IBS) is a functional GI disorder characterized by abdominal pain and is diagnosed following the Rome Ⅲ criteria. Multiple factors are involved in the pathophysiology of IBS; amongst others IBS has been associated with abnormal GI motor functions, visceral hypersensitivity, as well as psychosocial factors[100,101].

Some studies suggest a possible role of melatonin in the pathophysiology of IBS. For example, disturbances in melatonin metabolism and secretion may be involved in different GI diseases including IBS[102]. In a clinical trial involving patients with IBS, the beneficial effects of melatonin were obvious in the relief of symptoms such as abdominal pain, abdominal distension and abnormal sensation of defecation[103]. Melatonin may exert its beneficial effects in IBS through effects on the central nervous system, *via* an enhancement of the cellular and humoral immune systems, or by antagonizing corticoid- and serotonin-mediated effects[104,105]. However, melatonin does not influence sleep pattern or psychological well-being in patients with IBS. Recently, it has been shown that the antinociceptive effects of melatonin are not mediated through melatonin receptors, but through a supra-spinal process linked to the central opioidergic system, as pre-treatment with naltrexone or luzindole blocked the antinociceptive effect of melatonin in TNBS-treated rats[106].

According to recent clinical trials, melatonin may be a future therapeutic option for IBS management (Table 2). In one placebo-controlled, randomized clinical trial in 40 patients with IBS, daily administration of melatonin 3 mg orally at bedtime for two weeks significantly alleviated abdominal pain[107]. Patients treated with melatonin for two weeks significantly increased rectal thresholds towards balloon pressure and volume, ameliorating rectal sensitivity to pain and urgency. In another clinical trial, 17 female

Table 2 Clinical trials using melatonin in patients with irritable bowel syndrome

Authors	*n*	Study design	Dose	Conclusion
Lu *et al*[110]	17	Randomized, crossover placebo-controlled (8 wk)[1]	3 mg/*od*	CTT did not change significantly in IBS patients with melatonin treatment
Saha *et al*[109]	18	Randomized, placebo-controlled (8 wk)[1]	3 mg/*od*	Significant symptomatic benefit on bowel symptoms, extracolonic symptoms, and quality of life
Lu *et al*[108]	17	Randomized, crossover placebo-controlled (8 wk)[1]	3 mg/*od*	Significant symptomatic benefit on IBS scores, anxiety, well-being, and depression scores
Song *et al*[107]	40	Randomized, placebo-controlled (2 wk)[1]	3 mg/*od*	Significantly attenuated abdominal pain and reduced rectal pain

[1]Treatment duration; *od*: Once daily; CTT: Colonic transit time; IBS: Irritable bowel syndrome.

IBS patients were randomized to receive either melatonin 3 mg or placebo at bedtime for 8 wk, followed by a 4-wk washout period[108]. Improvements in mean IBS scores were significantly greater during treatment with melatonin compared to placebo. Additionally, sleep, anxiety and depression scores improved. Saha *et al*[109] randomly assigned 18 IBS patients to receive either melatonin 3 mg or placebo at bedtime for 8 wk and they found that melatonin significantly improved overall IBS scores and quality of life scores. All these trials suggest that melatonin has beneficial effects in patients with IBS, but larger clinical trials in patients with IBS are needed. Another clinical trial was interested in colonic transit time (CTT) in IBS patients. These patients were randomized and received either melatonin 3 mg or placebo daily for 8 wk[110]. Neither in healthy controls, nor in IBS patients, were stool texture or CTT changed, but the tests used may not be sensitive enough to detect motility or secretory changes and thus these data need to be interpreted cautiously.

In some of these clinical trials it has been shown that the beneficial effects of melatonin in IBS may be related to its action on gut sensory pathways. In this context it would be interesting to know whether melatonin alters visceral hypersensitivity or whether it acts as a general analgesic and would reduce rectal sensations in healthy volunteers as well. It is presently not clear whether this melatonin effect on rectal sensation is short-lasting or holds over longer periods of time. Although only a few clinical studies have shown its efficacy, melatonin appears to have a significant role in reduction of abdominal distension and rectal pain in treatment of IBS.

Inflammatory bowel disease

Despite the numerous animal studies showing protective effects of melatonin in colitis models, there are only limited clinical data available on the therapeutic role of melatonin in inflammatory bowel disease (IBD). To our knowledge, there are three published case reports of the self-administration of melatonin in IBD (Table 3). However, no clinical trials have been performed in IBD patients.

In one case report, after the self-administered use of melatonin as a self directed treatment for jet lag on international flights, the patient observed that his ulcerative colitis (UC) symptoms were virtually absent[111,112]. Once his flare-ups were more troublesome requiring continuous topical mesalamine therapy, he self-administered melatonin 3 mg/d, and according to the report he was symptom-free for a period of 3 mo. His symptoms recurred within 1 wk of running out of melatonin tablets. In contrast, other cases showed melatonin exacerbated symptoms associated with UC or Crohn's disease[113,114]. One patient decided to take melatonin capsules (3 mg) at bedtime. Two months later, the patient started to experience the symptoms of active UC, including bloody mucous diarrhea. He continued taking melatonin and received corticosteroids orally and rectally. Since the symptoms did not calm down, the patient was hospitalized and stopped consuming melatonin; 48 h later there was a complete remission of the UC symptoms. Another patient decided to take melatonin capsules (3 mg) at bedtime. Four days later, the patient started to experience the symptoms of active Crohn's disease, such as diarrhea and abdominal cramps. She then stopped taking melatonin, and 24 h later there was a complete remission of symptoms. Clinical trials should be performed to evaluate a possible beneficial or detrimental effect of melatonin in IBD; presently available literature is inconclusive, though basic studies strongly suggest beneficial effects.

Colon cancer

Following the identification of melatonin binding sites in human colon tissue from patients with carcinoma of the rectum and the colon, a possible role of melatonin in colorectal cancer was addressed in several studies. ^{125}I-melatonin binding sites were identified in the mucosa and the submucosa of the human colon and radioimmunoassays revealed melatonin concentrations of 467 ± 99 pg/g tissue in non-cancer control patients, while daytime melatonin concentrations in the colon of patients with colorectal carcinoma were 3147 ± 87.8 pg/g tissue[115]. The relevance of the diurnal variation of melatonin levels to colon cancer has yet to be determined. Colorectal carcinoma patients showed significant decrements in the peak amplitude of melatonin secretion, as well as a reduction in overall melatonin output[116]. Some studies suggest that melatonin may be involved in cancer risk or protection from cancer development[117]. For example, following pinealectomy, increased colonic crypt cell proliferation was reported in rats, suggesting melatonin pathways being involved in carcinogenesis in the co-

Table 3 Case reports of melatonin self administration in patients with ulcerative colitis and Crohn's disease

Authors	Age, gender, disease	Treatment	Dose	Result
Maldonado *et al*[113]	56, male, UC	Added melatonin to the otherwise unchanged drug treatment (salazosulfapyridine, corticosteroids)	3 mg/*od*	Two months later, the patient started to experience the symptoms of active UC, including bloody diarrhea
Jan *et al*[111] Mann[112]	47, male, UC	Added melatonin to an existing medication of mesalamine due to ongoing bloody diarrhea	3 mg/*od*	Symptoms resolved fast (2–3 d) and the beneficial effect was long lasting
Calvo *et al*[114]	35, female, CD	After becoming pregnant, the patient interrupted the treatment with melatonin, corticoids and salazosulfapyridine and symptoms of CD emerged again	3 mg/*od*	Recurrence of diarrhea and abdominal cramps within 4 d

od: Once daily; UC: Ulcerative colitis; CD: Crohn's disease.

lon[118]. Another study in rats showed that small bowel crypt cell hyperplasia occurred several weeks after pinealectomy, but again the exact mechanisms were not identified. Recently, melatonin showed a great potential to control the preneoplastic patterns induced by constant light in the colon[119].

The suggested colon cancer controlling mechanism of melatonin involves inhibition of tumour angiogenesis, modulation of the mitotic and apoptotic indices, and maintenance of the intracellular level of glutathione[120,121]. Although no effects of melatonin on *in vitro* cell growth were found, a statistically significant and progressive suppression of *de novo* DNA synthesis was found following melatonin application[122]. Other melatonin effects related to the control of tumour growth are the modulation of estrogen receptors, direct effects on the cell cycle, influence on several growth factors, increasing of gap junctions and enhancing the level of antioxidants[123,124]. The anti-oxidative and anti-inflammatory actions of melatonin, changing the oxidative status and reducing the production of nitric oxide by cultured colon cancer cells, may also be directly involved in the oncostatic properties of melatonin[125]. Some studies suggest that for colon adenocarcinoma, membrane-bound and nuclear melatonin receptors are involved in these oncostatic actions[126,127]. Melatonin binds to receptors on T helper cells and monocytes, stimulating the production of IFNγ and interleukins 1, 2, 6 and 12, which in turn up-regulates immune responses resulting in a restoration of immunodeficiency states[128]. Melatonin in this context also modulates the expression of NF-κB, TNF-α, IL-1β and STAT3[129]. The activation of lymphocytes and monocytes/macrophages by melatonin is one of the mechanisms by which melatonin as an immunosurveillant prevents tumor development[130,131]. For example, patients with advanced GI carcinoma treated with a combination of IL-2 and melatonin exhibited a significantly higher number of lymphocytes, T lymphocytes, NK cells and $CD4^+$ cells than those receiving IL-2 alone[132].

In clinical trials, melatonin was shown to have cytoprotective effects that may be involved in increasing the efficacy of cancer chemotherapy and improving survival. Melatonin co-treatment was also shown to reduce the adverse toxicities of chemotherapy and radiotherapy in several studies, including in patients with colorectal carcinoma[133,134]. For example, the efficacy of weekly low-dose CPT-11 in pretreated metastatic colorectal cancer patients may be enhanced by a concomitant daily administration of melatonin (20 mg/d, orally)[135]. Other clinical studies showed that melatonin co-treatment with IL-2, *Aloe vera* or fish oil partly enhanced the effect of chemotherapy and reduced the toxicity in colorectal carcinoma[136-139]. However, melatonin did not have any protective effect on irradiation-induced lymphocytopenia in patients with colorectal carcinoma (Table 4)[140].

Clinical trials using melatonin in the context of colorectal cancer are small and unfortunately not of high quality. Presently, these studies have to be carefully interpreted and the studies seem, if anything, to be hypothesis-generating. Controlled clinical trials are needed to establish the potential role of melatonin in cancer treatment.

CONCLUSION

Melatonin found in the lower gut comes largely from intestinal sources, such as the EC cells and, to only a minor extent, from extra-intestinal sources such as the pineal gland. Melatonin levels in the ileum and the colon are dependent on food intake and digestion, but in contrast to systemic melatonin levels, the GI melatonin level is independent of light or the circadian rhythm.

Melatonin regulates the motility of the lower gut by acting on membrane melatonin receptors and all known MT1-3 were found to be localized in the GI tract, though their exact involvement in the regulation is not fully characterized. Additionally, actions of melatonin on 5-HT receptors have been reported, adding to the complexity of melatonin involvement in the regulation of GI function.

Melatonin was recently suggested to be a promising future drug for IBS treatment. Presently available basic and clinical data indicate that it is particularly effective in alleviating hypersensitivity and pain in patients with IBS, but larger clinical trials, ideally double-blinded and placebo-controlled, are needed.

Melatonin is furthermore involved in immunomodulatory functions throughout the GI tract. The protective actions of melatonin in mouse models of intestinal inflammation or in models of GI cancer are promising

Table 4 Clinical trials using melatonin in patients with colorectal cancer

Authors	*n*	Disease	Study design	Dose	Results and conclusion
Lissoni *et al*[140]	18	Rectal cancer	Randomized to melatonin, melatonin + 5-methoxytryptamine or melatonin + IL-2, 5 wk	20 mg/*od*	Melatonin had no effect on radiation-induced lymphocytopenia
Lissoni[133]	152	CRC	Randomized to oxaliplatin/5-Fu or CPT-11/FS/5-Fu with or without melatonin	20 mg/*od*	Melatonin significantly reduced the occurrence of cachexia, thrombocytopenia, neurotoxicity and asthenia
Persson *et al*[139]	8	CRC	Randomized to fish oil or melatonin (4 wk) followed by 4 wk fish oil with melatonin	18 mg/*od*	Melatonin had no effect on serological inflammation markers
Cerea *et al*[135]	30	CRC	Randomized to CPT-11 or CPT-11 plus melatonin 9 wk	20 mg/*od*	Disease-control higher in CPT-11 + melatonin group
Lissoni *et al*[120]	7	CRC	Daily melatonin for at least 2 mo	20 mg/*od*	Melatonin may control tumor growth by reducing VEGF secretion
Lissoni *et al*[134]	25	CRC	Randomized to 5-Fu/FS or 5-Fu/FS + melatonin. 5 cycles of 28 d	20 mg/*od*	Melatonin reduces toxicity and increases efficacy of 5-Fu/FS chemotherapy
Lissoni *et al*[138]	8	CRC	Randomized to melatonin or melatonin + *Aloe vera* tincture until progression	20 mg/*od*	Melatonin + *Aloe vera* stabilized disease and increased survival in end-stage patients
Barni *et al*[137]	50	CRC	Randomized to BSC or BSC combined with low-dose IL-2 + melatonin 4 wk	40 mg/*od*	Low-dose IL-2 + melatonin induced tumor regression and prolonged survival in second-line treatment
Lissoni *et al*[136]	19	CRC	Randomized to IL-2 or IL-2 + melatonin 4 wk	40 mg/*od*	Melatonin enhanced the activity of IL-2, induced tumour regression, prolonged progression-free survival and overall survival

CRC: Colorectal cancer; *od*: Once daily; BSC: Best supportive care; VEGF: Vascular endothelial growth factor.

and warrant further research. The translation of these observations to humans is less well characterized.

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