Review

Functional interplay of melatonin in the bile duct and gastrointestinal tract to mitigate disease development: An overview

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ABSTRACT

Prevalence of bile duct and gastrointestinal (GI) tract associated diseases is increasing globally. Commonly, the bile duct epithelial cell (cholangiocytes) malfunction and its uncontrolled proliferation often cause liver fibrosis and tumorigenesis, particularly the cholangiocarcinoma. Specifically, GI tract is constantly under diverse endogenous and exogenous stressors which interrupt GI physiological functions and promote inflammation, tissue damage, ulceration, gastrointestinal bleeding, gastroesophageal reflux disease (GERD), irritable bowel disease (IBD) and gastritis. On the other hand, melatonin exhibits important functions in both cholangiocyte and GI tract. The abundance of melatonin generated in the GI tract and its widely distributed receptors facilitate its protective effects in GI tissues. In the most of the cases, the disease progression in GI tract, particularly in bile duct, is associated with endogenous melatonergic system suppression. Therefore, to increase the endogenous melatonin production appears a suitable strategy to retard the disease development in these tissues. Melatonin administration or, exposure to prolonged darkness not only reverse the detrimental biochemical alterations, but also inhibit cholangiocyte proliferation as well as ulceration in the GI tract. Thus, use of melatonin as a natural therapeutic agent is beneficial and exhibits advantages over other contemporary drugs in prevention and treatment of bile duct and gastrointestinal tract associated diseases.

Key words: Bile duct, cholangiocytes, gastrointestinal tract, diseases, melatonin, prevention.

1. INTRODUCTION

In humans, interruption of bile metabolism leads to cholestasis that is considered as a hallmark of different liver disorders (1). Over production of particular bile acids will cause structural damage, inflammation, fibrosis and tumorigenesis in hepatic tissue, and induce bile duct proliferation as well (2). Thus, it is important to understand the functionality of the biliary system during any pathogenesis. It is well known that the response of cholangiocytes, epithelial cells lining the intra- and extrahepatic bile duct, plays an important role during the development of various biliary system associated disorders, such as cholestatic liver diseases (3, 4). Although the exact mechanism of cholangiopathy is not understood to date, a preliminary ductular response induced biliary hyperplasia is known to trigger apoptosis, ductopenia and fibrosis (5). Normally, cholangiocytes remain quiescent but any sort of stress or tissue injury

in the liver will activate the proliferative characteristics of the cholangiocytes. This response is regulated by a complex network of different proteins and transcription factors (6, 7). This ductular reaction is actually a critical interplay between cholangiocytes and different liver cells during the development of cirrhosis (4). Physiologically, gastrointestinal (GI) tract is the primary battle ground since pathogens directly interact with its epithelial lining to trigger inflammatory reaction (8). The reactive oxygen species (ROS) generated by pathogens or internal stressors can cause numerous pathophysiological alterations including malignancy (8). In addition, GI damage can also be caused by several other factors such as heavy metals (9, 10), ischemia-reperfusion (11), alcohol consumption (12), non-steroidal anti-inflammatory drugs (NSAIDs) (13, 14) and high fat diet (8, 15). Notably, high level of bile acids can cause gastroesophageal reflux disease (GERD) by the mechanism of elevated ROS (16). Bile acids are also the strong cyclooxygenase-2 (COX-2) inducers in case of Barrett's esophagus and esophageal adenocarcinoma with increased ROS production and activated PA3K/AKT and ERK1/2 pathways (17). These important observations clearly suggest the possible functional interplay between the biliary system and GI tract during pathogenesis.

Several approaches have been adopted so far to overcome these pathophysiological conditions related to the biliary system in GI tract. These include use of some neuro-endocrine hormones, neurotransmitters, and growth factors which show promising effects in inhibiting the detrimental changes induced by the stressors (18-20). Among these, melatonin is considered as the most promising agent due to its lipophilic nature, paracrine actions, broad spectrum antioxidant and direct free radical scavenging properties (21-26). High levels of melatonin presence in bile (27) and GI tract (25, 28, 29) of mammals including humans (30) provide a unique advantage to overcome many pathological conditions in these tissues. In this review, we will focus on the beneficial effects of melatonin in combating against biliary and GI pathogeneses in mammals.

2. STRUCTURAL FEATURES OF BILIARY EPITHELIUM AND ITS DUCTAL SYSTEM

Among the different hepatic epithelial cells, hepatocytes and cholangiocytes play the critical roles in the regulation of biliary secretion and intra-hepatic functional homeostasis (3, 31). Anatomically, the intra-hepatic bile ductal apparatus forms the tubular structures that are interconnected and lined by diverse cholangiocytes (3, 32, 33). In humans, the intra-hepatic biliary epithelium has been classified into six categories (33) based on the diameter of the bile duct- (i) small bile ductules (<15 μm), (ii) intra-lobular ducts (15-100 μm), (iii) septal ducts (100-300 μm), (iv) area ducts (300-400 μm), (v) segmental ducts (400-800 μm) and (vi) hepatic ducts (<800 μm). However, the biliary epithelium of rodent has been classified into two types- (a) large (>15 μm) bile ducts that are lined by large cholangiocytes while (b) small (<15 μm) bile ducts that are lined by smaller ones (34-36). The intra-hepatic cholangiocytes comprise 3-5% of the entire liver cell population (3). In addition to cholangiocyte-regulated autocrine loop, the branches of hepatic artery form a complex mesh of minute vessels known as the peri-biliary vascular plexus (PBP) that secretes numerous growth factors, such as vascular endothelial growth factor (VEGF), are responsible for the regulation of the bile duct system (37, 38). Hepatic sinusoids are directly supplied through the lobular branches or, indirectly via the pre-lobular portal vein branches (37, 39). Usually, PBP is profoundly distributed around the large bile ducts but is present in lesser number in the smaller bile ducts (37).

At this point it is important to discuss the path of progression of the secreted bile. Initially, bile is secreted into the canalicular region from where it travels through the Hering canals to reach to the small ductules (31). Eventually, large bile ducts collect the canalicular bile by re-absorption and secretion (35, 40, 41) that are under the control of different parasympathetic,

sympathetic and dopaminergic innervations as well as numerous gastrointestinal hormones (for example secretin, gastrin and somatostatin) (35, 40-45). Interestingly, the properties of such re-absorption and secretion related to the biliary epithelium vary according to the size of the bile ducts and its position in the biliary tree (34, 35, 41, 46). For instance, among the diverse hepatic cell types, secretin receptor (SR), cystic fibrosis trans-membrane conductance regulator (CFTR), somatostatin receptor subtype 2 (SSTR2), and $Cl^-/HCO3^-$ anion exchanger AE2 are solely found in the large colangiocytes (34, 35, 42, 47); this explains the possible cause of why large cholangiocytes can only respond to secretin mediated stimuli to regulate the release of water and electrolytes (34, 35, 42, 47). In contrast, small cholangiocytes within the small ducts does not express any of these receptors or transporter proteins and thereby remains inactive to secretin and somatostatin stimulations (34, 35, 42, 47).

3. FUNCTIONAL DIVERSITY OF CHOLANGIOCYTES

The size variation of cholangiocytes actually reflects its functional diversity in mammals (34, 48). The large cholangiocytes which are responsible for the secretion of water and bicarbonate are dependent on 3', 5'-cyclic adenosine monophosphate (cAMP) regulation (34). In contrast, small cholangiocytes are inositol trisphosphate/Ca^{2+} dependent ones. Under normal condition, these small cholangiocytes serve as a silent progenitor which then, can differentiate into large cholangiocytes after cellular injury (48). The active transportations of glutathione and organic anions such as bilirubin glucuronide into the canaliculus, a phenomenon known as bile acid-independent bile flow (49), occur in large cholangiocytes since they possess secretin specific receptors (SRs) on their baso-lateral membranes (40, 50). Secretin binds to SR to elevate the intracellular levels of cAMP which in turn, activates cystic fibrosis trans-membrane conductance regulator (CFTR). These events result in the leakage of Cl^- to extracellular side causing activation of the Cl^-/HCO_3^- exchanger AE2, thus triggering bicarbonate-rich choleresis; however, Cl^- is restored in cholangiocytes (40).

Apart from its crucial physiological roles, cholangiocytes also exhibit potent protective effects against the diverse biliary disorders including primary sclerosing cholangitis (3), and symptoms associated with fibrosis and cirrhosis (4). Otherwise, the malfunctioned cholangiocytes will promote biliary hyperplasia which in turn causes apoptosis, ductopenia, and fibrosis (5).

4. INJURY INDUCED MODULATION OF CHOLANGIOCYTE'S FUNCTIONS

During the initial stages of injury, sensitivity of cholangiocytes to a variety of neuroendocrine peptides (secretin), neurotransmitters (histamine), growth factors [nerve growth factor and vascular endothelial growth factor (VEGF)] and hormones [follicle-stimulating hormone (FSH), estrogen and progesterone] increases in a neuroendocrine like manner that eventually triggers biliary proliferation, liver inflammation and fibrosis (6, 7, 38, 51-55). Some of these transdifferentiation inducing factors are discussed herein. In case of cholestatic hepatic injury, proliferative response of cholangiocytes occurs due to the suppression of the hypothalamic-pituitary-adrenal axis. This is indicated by the increased hypothalamic bile acid signalling, suggesting the involvement of other neural hormones in the regulation of cholangiocyte mediated actions (56, 57).

Abundance of GnRH receptors (GnRHR1 and GnRHR2) found in cholangiocytes indicates regulatory role of GnRH in these cells (58). This is also supported by the observation that bile duct ligation (BDL) increases the levels of GnRH in the cholangiocytes and triggers their proliferation (58). Generally, GnRH level varies between 0.05-0.06 ng/mL in the supernatant of normal mouse cholangiocyte cell line (58). Treatment with recombinant GnRH enhanced

intrahepatic bile duct mass due to increasing cholangiocyte proliferation (57). Several molecules can modify the proliferation of cholangiocytes. For example, melatonin can regulate cholangicyte proliferation by suppressing the expression of hypothalamic GnRH through different protein kinase pathways (59-60). Sex hormones such as FSH are also involved in biliary hyperplasia possibly by triggering proliferation of cholangiocyte, increasing ductal mass and intracellular levels of cAMP, inducing phosphorylation of both ETS domain-containing protein and membrane bound kinases (61). Another important factor is pancreatic duodenal homeobox-1 which is over expressed in the isolated cholangiocytes of BDL rat. In pancreatic duodenal homeobox-1 deficient mice, their bile duct mass, VEGF and insulin-like growth factor 1 are reduced which are biomarkers of cholangiocyte proliferation (62). In addition, secretin/secretin receptor axis also plays potent roles in regulating cholangiocyte responses (63). Physical damage to cholangiocyte suppresses the expression of SR in large cholangiocyte, whereas basal cholangiocyte proliferation is completely inhibited by SR knockdown (54). These findings indicate the importance of secretin/SR as an essential tropic regulator of biliary growth in mammals (54). Histamine receptors are other regulators for cholangiocyte proliferation. All four types of histamine receptors (H1HR, H2HR, H3HR and H4HR) are present in cholangiocyte. H3HR and H4HR exhibit inhibitory while H1HR and H2HR exhibit stimulatory actions to maintain the balance of cholangiocyte proliferation (64-67). Notably, application of H3HR agonist decreased BDL-induced cholagiocyte hyperplasia in rats by targeting the cAMP signalling along with phosphorylation of ERK1/2 and transcription factor E1k1 pathway (64). In contrast, activation of H1HR induces proliferation of small cholangiocytes through IP3/Ca2+/PKC/CaMKI/CREB-dependent pathway (64). Application of H4HR agonist (clobenpropit) restricts cholangiocarcinoma growth and metastatic potential through activation of a $Ca^{2+/}$PKC-dependent pathway and by reducing the expression of matrix metalloproteinases (67). Noteworthy, VEGF and its receptors (VEGFR-2 and VEGFR-3) are present in cholangiocytes. In BDL animals, both VEGF level and VEGF receptor expression are enhanced with increased proliferation which are mediated by activation of IP3/Ca^{2+}/PKCα and Src/ERK1/2 signalling (68). Both subtypes of estrogen receptor (ER-α and ER-β) are also expressed in cholangiocytes to facilitate the proliferative and secretory activities of cholangiocytes. In BDL animals, expressions of ER-a and ER-b were upregulated from 3 to 30 folds, respectively, compared to the controls (69). This is also observed in the *in vitro* study, in which 17β estradiol stimulates cholangiocyte proliferation (69). Usually, the nuclear (PR-B) and membrane (PRGMC1, PRGMC2, and mPRα) progesterone receptors are expressed in cholangiocytes. Progesterone exerts its autocrine/paracrine actions on cholangiocyte proliferation (70). In BDL rat, antiprogesterone antibodies efficiently restricted the proliferation of cholangiocytes (70).

5. BILIARY SYSTEM ASSOCIATED DISORDERS

In diverse cholangiopathy models, extrahepatic bile duct ligation (BDL), acute carbon tetrachloride (CCl_4) treatment and chronic gamma-aminobutyric acid (GABA) administration are a few of them which alter the secretory and proliferative activities of cholangiocytes (48, 61, 71). Under these treatments, cholangiocytes usually lose their differentiation capability and reduce sensitivity to hormone intervention with increased biliary apoptosis (48, 61, 71). When an injury occurs in large bile ducts, the small cholangiocytes amplifies Ca^{2+}-dependent signalling pathways to restore the biliary epithelium followed by Ca^{2+}/CaMK I-dependent adenylyl cyclase 8 activation and appearance of large cholangiocyte phenotype (48, 61, 71). On the other hand, cholangiopathies such as primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and cholangiocarcinoma (CCA), if not handled properly, will cause liver inflammation, ultimately hepatic fibrosis (72, 73). For example, PBC and PSC

associated bile duct proliferation, commonly termed as the ductular reaction (DR), is an essential step of disease development where non-functional truncated bile ducts invade into the parenchymal region of liver through the portal areas (52, 74, 75). Suppression of DR is linked with reduced liver fibrosis. This might be a collective resultant of declined response in biliary secretory phenotypes and hepatic stellate cell activation factors (76). Bile acid malabsorption (BAM) induced chronic diarrhoea is another area of concern since the absorbed chenodeoxycholic and deoxycholic acids, major components of dihydroxy bile acid, are known to restrain sodium absorption in the colon but triggers chloride secretion, ultimately leading to diarrhoea (77).

Several other physiological factors are also involved in biliary system complications. For example, excess production of mitochondrial reactive oxygen species (mROS) in extrahepatic cholestatic patients causes oxidative damage to different mitochondrial components, such as unsaturated fatty acids, proteins and DNA (78). Glycochenodeoxycholic acid (GCDCA), the predominant compound in the bile, seems to be responsible for the development of extrahepatic cholestasis (79) where lipid peroxidation of the mitochondrial membrane plays the pivotal role (79, 80).

6. GASTROINTESTINAL TRACT ASSOCIATED DISORDERS

As mentioned above, GI tract is always under the endogenous and exogenous insults (23, 25, 26, 81). For example, the endogenously produced adrenaline, due to its rapid pro-oxidant and auto-oxidation properties (82-84), promotes oxidative stress and proinflammatory cytokines including necrosis factor-kappa beta (NF-kβ), tumour necrosis factor-α (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL6) in the stomach, duodenum and colon tissues (25). The environmentally extracted heavy metals such as arsenic (As), lead (Pb) and mercury (Hg) also cause detrimental changes in the GI tract (10, 85, 86). These metals bind to sulfhydryl group of enzymes to interrupt their functions and increase the production of superoxide anion, hydrogen peroxide and hydroxyl radical which in turn elevates peroxidation of membrane lipids and apoptosis (86). The monomethylarginine (MMA) compounds, the products of interaction of As and thiol group of nitric oxide synthases, cause diverse GI symptoms termed as arsenicosis (86). Similarly, Hg causes gastric ulceration and hemorrhage (85), abdominal pain, vomiting and bloody diarrhoea (87). Pb is another disease promoting agent in the GI tract (88, 89). Pb mimics some co-factors, such as calcium, iron and zinc, to catalyze different intracellular enzymatic reactions and activities of varied pro-oxidant enzymes (90) to increase the threat of stomach cancer in humans (10).

The non-steroidal anti-inflammatory drugs (NSAIDs) can also cause diverse pathogenesis in the GI tract (8, 91). Basically, NSAIDs promote generation of ROS and inhibit mucosal blood flow, collectively to trigger the gastric ulceration and inflammation (92, 93). NSAIDs, such as aspirin, indomethacin and ibuprofen inhibit prostaglandin production and cellular differentiation possibly by exhaustion of ATP supply due to disruption of mitochondrial transmembrane potential and increasing permeability of transition pore. The dysfunctional mitochondria generate more hydrogen peroxide and hydroxyl radical leading to oxidative damage in the gastric mucosa (92, 94-97). Indomethacin and piroxicam cause irreversible inactivation of gastric peroxidases, downregulate antioxidant enzymes and increase lipid peroxidation, thus promoting over production of ROS and ulcerative symptoms in the GI tissues (98, 99). Ischemia reperfusion (I/R) also induces severe inflammation and tissue injury in the mammalian GI tract (11). Profound accumulation of activated neutrophils and over production of ROS during reperfusion further exacerbates the GI injuries (100, 101). The sepsis and ulceration are also noted in patients with ischemic colitis (102). Noteworthy, consumption of high fat diet (HFD) induces inflammation in mammalian GI tract (15) possibly by altering

the intestinal levels of chylomicrons and gut microbiota sub-colonies (103). Similarly, alcohol consumption also affects the intestinal microbiome and inhibits bile acid metabolism (104), triggers over production of hydrogen peroxide and carbon-centred radicals which in turn lead to endothelial dysfunction, microcirculatory disturbance and ischemia of gastric mucosa (21). Moreover, some bacteria, such as *Helicobacter pylori,* are the culprits of peptic ulcer disease, gastroesophageal reflux disease (GERD), irritable Bowel disease (IBD) and gastritis. These pathogens activate non-phagocytic NADPH oxidase to promote proinflammatory responses, oxidative damage to the gastric epithelial pit cells (8, 105, 106). The development of duodenal ulcer and severe duodenitis have been observed in humans infected by *H. pylori* (107).

7. MELATONIN: A POTENT THERAPEUTIC MOLECULE

The protective effect of melatonin on biliary system disorders were observed in the BDL study in which pinealectomy and prolonged exposure to light exacerbate biliary damage and liver fibrosis in rats. Both treatments reduce melatonin level, and thus, increase ROS and inflammation with upregulation of expression of CLOCK, ARNTL, Cry1, and Per1, and miR-200b clock genes in cholangiopathies (108). The potent antioxidant and cellular permissible properties lead melatonin having advantages over other antioxidants in protecting cholangiocytes oxidative damage (21, 109, 110). Melatonin upregulates antioxidant enzymes and downregulates pro-oxidant enzyme expressions to exhibit its indirect antioxidant actions (22, 24, 25). This action is mediated by the activation of its membrane bound receptors (MT1R, MT2R and MT3R) (111). In addition, its metabolites N^{l}-acetyl-N^{2}-formyl-5-methoxykynuramine（AFMK）, N^{1}-acetyl-5-methoxy-kynuramine (AMK) and cyclic 3-hydroxylmelatonin (C3HM) are also known to exert strong antioxidant and anti-inflammatory activities (112). Melatonin also maintains the cellular energy homeostasis by targeting mitochondria and prevents bile-acid induced oxidative damage in the hepatocytes through modulation of the AMPK-SIRT3-SOD2 pathway in liver (25, 113-116). All these contribute to the beneficial effects of melatonin on cholestatic liver injury (117, 118).

8. MELATONINAND ITS METABOLISM

Melatonin is mainly metabolized in the liver through a two-step mechanism. First, cytochrome P450 mono-oxygenases hydroxylates C6 position forming 6-hydroxymelatonin; then, 6-hydroxymelatonin conjugates with (i) sulphate catalyzed by the sulphotransferase to form 6-sulphomelatonin or, (ii) glucuronic acid catalyzed by UDP-glucuronosultransferase to form 6-hydroxymelatonin glucuronide (119). In the second catabolic pathway, indolamine-2,3-dioxygenase (2,3-IOD) or myeloperoxidase catalyze melatonin forming AFMK which is later transformed to a more stable compound AMK (120, 121). Noteworthy, melatonin is also demonstrated to be metabolized non-enzymatically by the interaction with different ROS and RNS (21).

9. PRESENCE OF MELATONIN IN THE GI TRACT AND BILIARY SYSTEM

Concentration of melatonin in the bile is much higher than that in other organs/tissues. Melatonin levels in bile vary (ranging from 2,000 to 11,000 pg/ml) among the different mammalian species (27). Highest melatonin levels in bile were detected in rabbit (>10,000 pg/ml) followed by monkey (>8,000 pg/ml), human (>6,000 pg/ml) and rat (~4,000 pg/ml) (27). Such high level of melatonin in the human bile has protective effects on liver damage (122). Melatonin concentrations in the gastrointestinal tract are also relatively high. For example, the level is 467 ± 99 pg/g tissue in human (30) and 461± 29 pg/g tissue in rat (25).

There are some variations in different tissues including 136 ± 27 pg/100 mg in the GI mucosa and 243 ± 37 pg/100 mg in the stomach and descending colon, respectively (123). A study has reported a relative low melatonin level in bile (85 ± 45 pg/ml) and this may be due to its excretion into the GI tract via the bile duct (123). Immunolocalization and autoradiographic studies revealed the presence of melatonin in the enterochromaffin cells (EC) of the mucosa and villi of the gastrointestinal tract (28, 29, 124, 125). The discovery of melatonin synthesis rate-limiting enzymes, hydroxyindole-O-methyltransferase (HIOMT) and aralkylamine N-acetyltransferase (AANAT) in the EC cells confirm the *de novo* melatonin synthesis in the EC cells of the GI tract (25, 80, 110, 126, 127).

10. FUNCTIONAL RELEVANCE OF MELATONIN IN THE BILIARY TRACT

As yet cholangiopathies are considered as the prime cue for the development of hepatic failure and death in humans (128). Melatonin seems to be a suitable agent in the management of chronic cholestatic liver diseases because of its regulatory capability to maintain biliary homeostasis and to inhibit collagen deposition in the hepatic tissue (129).

Melatonin at a dose of 750 μg/kg BW/day suppressed the levels of transaminases, lipid peroxidation and glutathione in BDL rats and its protective efficiency was found to be superior to S-adenosyl-methionine (SAME, 10 mg/kg BW/day) (130). Melatonin receptor MT1 expression increased in the cholangiocytes isolated from the bile ducts of cholestatic rodents and melatonin (20 mg/L in drinking water) reduced serum transaminase and bilirubin and ductular reaction in the cholangiocytes (20). Melatonin treatment also inhibited the expression of clock genes, and levels of protein kinase A (PKA) phosphorylation and cAMP and these effects are probably mediated by its membrane receptor MT1 (20). In a separate experiment, upregulation of *AANAT* and increase in melatonin secretion were observed in isolated cholangiocytes of BDL rats (131).

On the other hand, natural light regimen was also applied to evaluate the role of melatonin through brain-liver axis in preventing the development of different cholangiopathies (76, 132). In order to upregulate the pineal secretion of melatonin, BDL rats and Mdr2-/- [possesses some characteristics of human primary sclerosing cholangitis (PSC)] mice are kept under complete darkness for 1 week. The results indicate that this treatment not only increases the melatonin levels in the pineal and circulation, but also improves the morphology of the hepatic tissue by decreasing collagen deposition and diminishes ductular reaction as well. Moreover, expressions of miR-200b, different clock genes, PER1, BMAL1, CLOCK and Cry1 are also declined (76, 133). The *in vivo* and *in vitro* studies on human PSC samples reveal similar responses when the studies are carried out in the complete darkness or, with melatonin treatment (76).

In contrast, circulating level of melatonin is found to be diminished in BDL cholestatic rats when they are subjected to pinealectomy or prolonged light exposure, accordantly, serum level of different hepatic enzymes, ductular reaction, and tissue levels of inflammation, angiogenesis, fibrosis, miR-200b and ROS generation are significantly increased (108). If the BDL rats are given intracerebroventricular (ICV) infusion of melatonin, ductular reaction and fibrosis of hepatic tissue are significantly reduced. These may relate to the suppressive effects of melatonin on GnRH expression and its secretion from the cholangiocytes along with a reduction in its receptor expression (133). Such observations are important as both biliary proliferation and fibrosis of liver are known to be triggered through GnRH and its receptor mediated pathway (134). Based on these evidences it may be assumed that enhancing pineal level of melatonin is beneficial in resisting the progression of cholestatic liver diseases.

11. FUNCTIONAL INTERPLAY BETWEEN BILIARY AND GASTROINTESTINAL MELATONIN

EC cells synthesize melatonin. A fraction of this melatonin is metabolized by cytochrome CYP1B1 in the enterocytes (135), a maximum amount (90%) of this melatonin enters the hepatic portal vein (136, 137) to reach the liver where it is metabolized (138) by hepatocytes by the cytochrome P-450 enzymes forming 6-sulfatoxymelatonin and 6-hydroxymelatonin glucuronide to be eliminated from the body through urine (139-141). However, some melatonin is released in the bile (27, 123) to prevent the epithelial lining of the bile and GI tracts from unwanted injuries induced by bile acids. Hepatic metabolic disturbances are also found to modulate the circulating levels of melatonin and its circadian rhythm (142-144). Many other instances have been reported to date that clearly suggested the possible functional correlation between the melatonin levels in the bile and GI tract (145).

12. MELATONIN PREVENTS GASTROINTESTINAL DISEASES

Gastrointestinal tract seems to be the richest source of endogenous melatonin in mammals where it regulates numerous local physiological actions mostly through its paracrine action (25, 26). In case of heavy metal toxicity, such as Cd-induced neurotoxicity melatonin exerts its protective role by activating transcription factor EB associated autophagic pathway (146) and restoring the equilibrium between mitochondrial fusion and fission (78, 147). The beneficial effects of melatonin against numerous heavy metal induced tissue damages have been frequently reported (148) and quite a few are dealt with GI tract. For example, exogenous administration of melatonin in rat prevents stomach and duodenum against Pb-induced oxidative injuries (149). Similar ameliorative actions of melatonin have also been reported against different NSAIDs, such as indomethacin, aspirin and piroxicam, induced intestinal tissue injuries (93, 119, 150-152). In case of diclofenac induced gastric damages, application of melatonin also restores mitochondrial membrane potential and energy metabolism, thus decreases intestinal permeability and maintains mucosal integrity (153, 154). The protection lies in the potentiality of melatonin in activating complex I and IV and inhibiting electron leakage, ultimately promoting mitochondrial respiration and ATP synthesis (97, 155, 156). Generally, melatonin is known to exert its gastro-protective efficacy by upregulating activities of different antioxidant enzymes, such as gastric peroxidise, SOD and catalase (157, 158). During GI ischemia-reperfusion, activation of MT2 receptor upregulates matrix metalloproteinase-2 (MMP-2) but downregulates MMP-9 and tissue inhibitor of metalloproteinases-2 (TIMP-2) to inhibit the oxidative tissue injury (159, 160). Apart from it, melatonin also prohibits gastric acid secretion and neutrophil infiltration and increases mucosal blood flow, duodenal level of bicarbonate secretion and prostaglandin production to inhibit intestinal tissue damage (161-163).

In GI disorders, such as GERD, use of melatonin and L-tryptophan (precursor of melatonin synthesis) as dietary supplement leads to regression of GERD symptoms (164). In water immersion and restrain stressed animal models, both melatonin and L-tryptophan protect GI tissue injury by increasing nitric oxide production and mucosal microcirculation (150, 165). Intragastric administration of melatonin (20 mg/kg) or tryptophan (100 mg/kg) caused a reduction in the lipid peroxidation level, while increasing the levels of SOD and GSH that clearly indicated the antioxidant role of melatonin (92). Melatonin also exerts its protective role against a number of stressors that induce serious gastric ulcers in mammals. In pig, application of melatonin (2.5 mg/kg/feed) in diet profoundly decreased the incidence of gastric ulcer (166). Since administration of melatonin or L-tryptophan upregulates MT2 receptor gene expression; therefore, the protective effects are attributed to its MT2 mediated pathways and

the enhancement of melatonin in ulcer bed speeds up the healing process (167). Combination of melatonin and ranitidine or omeprazole can produce synergic effects for the treatment of diverse types of gastric ulcers (157, 167, 168). Use of melatonin (20 mg per day) or tryptophan (500 mg per day) in *H. Pylori* induced gastric ulcer patients promoted their healing process (167). These may contribute to melatonin's antioxidant activity (22, 109, 111, 169, 170).

Interestingly, the metabolites of melatonin interaction with ROS also exert strong antioxidant functions thus amplifying the restoration process of the damages in GI tissues (171-173). Melatonin can also act on the melatonin receptors of the mast cells to produce its anti-inflammatory and immunomodulatory actions through inhibition of TNFα release, prostaglandin production and NF-κB expression (174-176). Similar anti-inflammatory (177) and antioxidant (178) roles of melatonin (25 mg/kg) are also observed in cerulein-induced acute pancreatitis in rats. The detailed mechanistic pathways have been described in Figure 1.

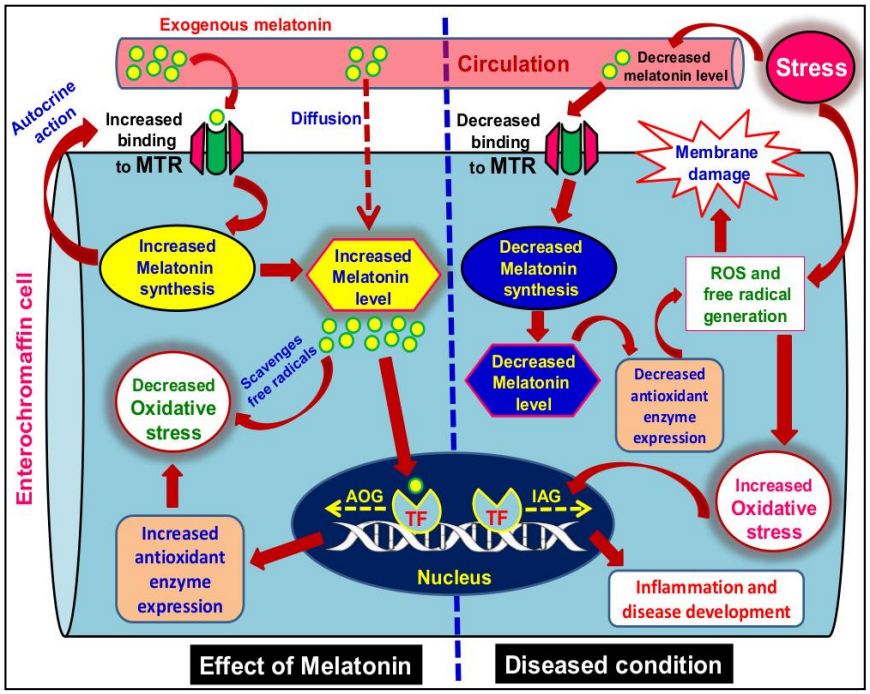

Fig. 1. The protective pathways of melatonin against GI disorders.

TF: Transcription factor; AOG: Antioxidant genes; IAG: Inflammation associated genes; MTR: Melatonin receptor (MT1 and MT2). ROS; Reactive oxygen species.

13. MELATONIN PREVENTS BILIARY TRACT DISORDERS

Current studies related to beneficial effects of melatonin on cholangiocytes are focused on the endogenous melatonergic system (130, 179). Decrease in *AANAT* expression and melatonin secretion in cholangiocytes of BDL rat causes biliary proliferation and increased activity of ductal secretion (130). In contrast, overexpression of *AANAT* restricts the proliferation of cholangiocytes (130). The α-naphthylisothiocyanate (ANIT), a potent biliary tissue toxin, induces hepatotoxicity which is prevented by oral melatonin (180, 181). The downregulation of *AANAT/ASMT* in patients with cholangiocarcinoma strongly suggests use of melatonin as a possible remedial agent (179, 182). As expected, treatment with melatonin inhibits the growth of *Opisthorchis viverrini* and N-nitrosodimethylamine-induced cholangiocarcinoma (179, 182). The potential mechanisms are summarized in Figure 2.

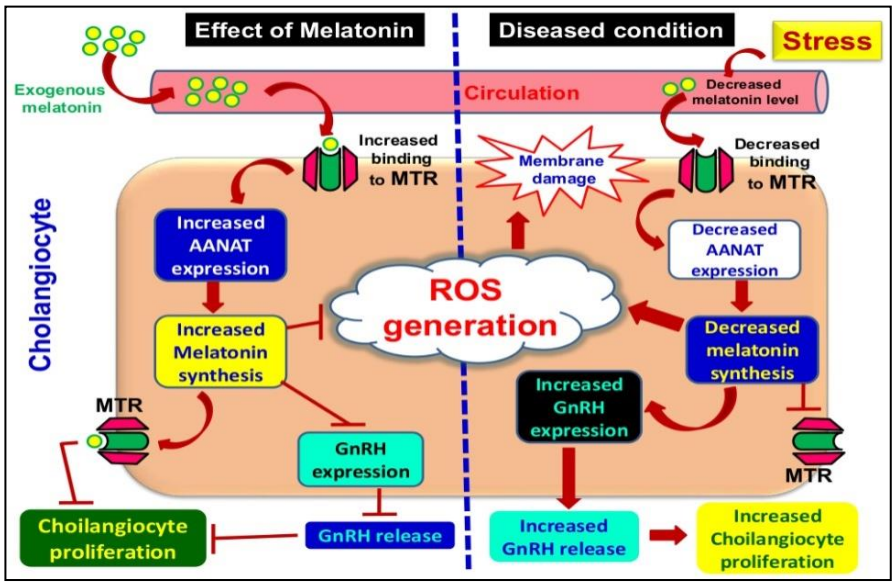

Fig. 2. The protective pathways of melatonin on disorders of bile duct epithelial cell (cholangiocyte).

MTR: Melatonin receptor (MT1 and MT2); ROS: Reactive oxygen species; GnRH: Gonadotrophin releasing hormone.

In addition to its peripheral effect, the role of melatonin on hypothalamic GnRH is important to combat cholangiocyte associated diseases since (i) GnRH secretion by cholangiocyte induces biliary proliferation and fibrosis (58, 131), (ii) melatonin can inhibit the activity of GnRH and suppress its secretion (183, 184), and (iii) exposing BDL rats to prolong darkness increases melatonin level in the cholangiocyte and reduces its proliferation (20, 131). Thus, the central suppressive effect of melatonin on secretion and expression of GnRH in the cholangiocyte will inhibit biliary proliferation and disorders. Actually, melatonin can inhibit GnRH secretion of hypothalamic cell in rat through modulation of different intracellular signalling pathways such as protein kinase A (PKA), protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) (59, 60). Similar antiproliferative action of melatonin in the cholangiocytes is also observed in different human CCA cell lines (Mz-ChA-1, HuH-28, TFK-1, CCLP1, SG231, and HUCC-T1) (179, 185). Study in rat hepatoma 7288CTC cells revealed that melatonin administration inhibited mitogenic signal for linoleic acid-dependent tumor growth by suppressing the absorption of linoleic acid and its further metabolism (186). Similar anticarcinogenic properties of melatonin are also documented in several human studies (187, 188).

The protective effects of melatonin on hepatotoxic compounds (30, 189, 190), ionizing radiation (191), cold storage and reperfusion (192-194), and carbon tetrachloride (195) are well documented. These protections are mediated either by its receptor dependent or receptor independent anti-inflammatory and antioxidant actions (196-200).

14. FUTURE PERSPECTIVES

Based on the above discussion it is clear that the functional relationship between melatonin levels in the bile duct and GI tract are important to prevent GI disorders. Thereby, use of melatonin as a natural therapeutic agent is beneficial and has advantages to other contemporary drugs as to treat biliary disorders since it lacks obvious side effects. Biliary proliferation induced by GnRH seems to be a key step in initiation of biliary pathology; therefore, the

inhibition of GnRH secretion will be a target for future drug development. Melatonin is a molecule to inhibit the GnRH secretion. Thus, melatonin alone or, as co-treatment with other medicines may be a future selection to combat diverse GI disorders.

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AUTHORSHIP

Dr. DB contributed to conception, revised the manuscript critically and approved it. Dr. AC revised the manuscript critically and approved it. Dr. PKP contributed to conception, prepared figures, drafted the manuscript and edited it.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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