

Melatonin regulation of biliary functions

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Abstract: The intrahepatic biliary epithelium is a three-dimensional tubular system lined by cholangiocytes, epithelial cells that in addition to modify ductal bile are also the targets of vanishing bile duct syndromes (i.e., cholangiopathies) such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) that are characterized by the damage/proliferation of cholangiocytes. Cholangiocyte proliferation is critical for the maintenance of the biliary mass and secretory function during the pathogenesis of cholangiopathies. Proliferating cholangiocytes serve as a neuroendocrine compartment during the progression of cholangiopathies, and as such secrete and respond to hormones, neurotransmitters and neuropeptides contributing to the autocrine and paracrine pathways that regulate biliary homeostasis. The focus of this review is to summarize the recent findings related to the role of melatonin in the modulation of biliary functions and liver damage in response to a number of insults. We first provide a general background on the general function of cholangiocytes including their anatomic characteristics, their innervation and vascularization as well the role of these cells on secretory and proliferation events. After a background on the synthesis and regulation of melatonin and its role on the maintenance of circadian rhythm, we will describe the specific effects of melatonin on biliary functions and liver damage. After a summary of the topics discussed, we provide a paragraph on the future perspectives related to melatonin and liver functions.

Keywords: Angiogenesis; biliary epithelium and growth; gastrointestinal hormones; liver damage; transduction pathways



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General background on liver functions

The liver is composed by several types of cells such as hepatocytes (parenchyma cells) and a non-parenchymal cell fraction that includes several cell types including intrahepatic bile duct cells (i.e., cholangiocytes), Kupffer cells, sinusoidal and vascular endothelial cells, hepatic stellate cells, dendritic cells, etc. Hepatocytes and cholangiocytes are the two hepatic epithelial cells that play key roles in biliary secretion and the maintenance of liver function/homeostasis (1,2). In rodents, hepatocytes comprise 70% of the total liver mass, whereas intrahepatic cholangiocytes form 3-5% of the total liver

cell population (1). The liver maintains the balance of the metabolism of the entire body synthesizing different types of proteins and enzymes. The liver also regulates the energetic metabolism, and it participates in the detoxification and elimination of a wide variety of xenobiotics (1).

Anatomical characteristics of the biliary epithelium

The intrahepatic bile ductal apparatus (that extends from the canals of Hering to the extrahepatic hepatic ducts) is

composed of interconnecting tubular structures that are lined by cholangiocytes of different diameters and functions (1,3-6). According to the Ludwig's classification (4), the human intrahepatic biliary epithelium has been defined according to bile duct diameter: small bile ductules (<15 μm in diameter), interlobular ducts (15 to 100 μm in diameter), septal ducts (100 to 300 μm in diameter), area ducts (300 to 400 μm in diameter), segmental ducts (400 to 800 μm in diameter), and hepatic ducts (>800 μm in diameter). Recently, we have begun defining the anatomical and morphological characteristics of the rodent biliary epithelium that we have classified as small (<15 μm diameter) and large (>15 μm diameter) bile ducts, lined by small and large cholangiocytes, respectively (3,7-9).

Vascularization of biliary ductal system

In addition to an autocrine loop regulated by cholangiocytes (10-12), the bile duct system is regulated by a number of growth factors [e.g., vascular endothelial growth factor (VEGF)] that are secreted by a complex network of minute vessels (i.e., peribiliary vascular plexus, PBP) originating from the branches of the hepatic artery (10,13). PBP flow mainly into the hepatic sinusoids, directly (lobular branch) or through the portal vein branches (prelobular branches) (10,14,15). A well-defined PBP is present around large bile ducts, but PBP is less visible around small bile ducts since it gets smaller proportionally to the size of bile ducts (10). In cholestatic rodents, concomitant with enhanced biliary hyperplasia there is proliferation of PBP, to support the nutritional and functional demand of the proliferating biliary epithelium (10). Consistent with the concept that cholangiocytes secrete angiogenic factors such as VEGF regulating biliary homeostasis by autocrine mechanisms, a study has shown that the proliferation of the PBP only occurs after that one of the bile ductal system (10).

General background on cholangiocytes

After secretion at the canalicular domain, bile reaches small ductules via Hering canals (2). Before reaching the small intestine, canalicular bile is modified by large bile ducts by a series of reabsorptive and secretory events (9,16,17) that are regulated by parasympathetic, sympathetic and dopaminergic innervation, gastrointestinal hormones such as secretin, gastrin, somatostatin and peptides (e.g., endothelin-1, ET-1) (9,16-24). The reabsorptive/secretory activity of the biliary epithelium is heterogeneous and depends on the size of the bile ducts and the anatomical

site within the length of the biliary tree (3,7,9,25,26). For example, we have shown that large cholangiocytes (lining large bile ducts) (7,9) are the only cell types in the liver to express secretin receptor (SR), the somatostatin receptor subtype 2 (SSTR₂), cystic fibrosis transmembrane conductance regulator (CFTR) and Cl⁻/HCO₃⁻ anion exchanger AE2 (7,9,20,27,28) and to respond to secretin with changes in the secretion of water and electrolytes (7,9,20,26,27). Conversely, small cholangiocytes in small ducts do not express SR, SSTR₂, CFTR and Cl⁻/HCO₃⁻ anion exchanger AE2 and do not respond to secretin and somatostatin (7,9,20,26-28).

In addition to heterogeneity with regard to secretory activity, cholangiocytes proliferate or are damaged differentially in a number of pathological models of cholestasis such as extrahepatic bile duct ligation (BDL), acute administration of carbon tetrachloride (CCl₄) and chronic administration of gamma-aminobutyric acid (GABA) (29-31). In these models, large more-differentiated cholangiocytes are more susceptible to these pathological maneuvers with loss of proliferative capacity, increase in biliary apoptosis and loss of responsiveness to hormones such as secretin (29-31). Following damage of large bile ducts, small cholangiocytes replenish the biliary epithelium by amplification of Ca²⁺-dependent signaling and acquisition of large cholangiocyte phenotypes by activation of Ca²⁺/CaMK I-dependent adenylyl cyclase 8 (29-31).

Background on melatonin

Melatonin [i.e., chemically as N-acetyl-5-methoxytryptamine, whose synthesis is mainly regulated by the rate-limiting enzyme, arylalkylamine N-acetyltransferase (AANAT)] (32), is a hormone found in animals, plants, and microbes (33,34). Melatonin is synthesized in the brain by the pineal gland from the amino acid tryptophan (35). The synthesis and release of melatonin are stimulated by darkness and reduced by light (detected by the photosensitive ganglion cells of the retina) (36), which suggest that melatonin plays a role in the modulation of the circadian rhythm and diverse body functions (32,35,36). In addition, melatonin secretion is also dependent on food consumption (37). A number of studies have shown that melatonin is produced in various extrapineal sites including bone marrow cells (38), lymphocytes (39), mast cells (40) and gastrointestinal tract including the biliary epithelium (41,42). The melatonin concentration in these cells is much higher than that found in the blood and contributes to the changes in melatonin concentration

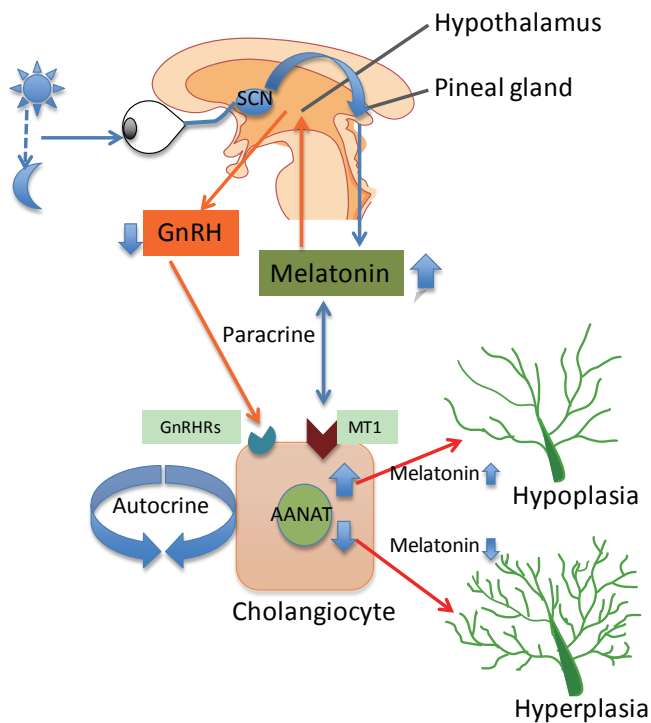


Figure 1 Melatonin production in the pineal gland is regulated by the light/dark signals that are detected by the retina. These signals pass through the retino-hypothalamic tract and the supra-chiasmatic nucleus to the pineal gland, where the melatonin is synthesized and secreted by pinealocytes. The release of melatonin from pineal gland interacts with melatonin receptors that are located in the hypothalamus to inhibit the production of GnRH. Inhibition of GnRH release may partly inhibit the biliary hyperplasia in cholestatic livers by interacting with the GnRH receptors. The release of melatonin from pineal gland inhibits cholangiocyte proliferation via interaction with the MT1 in a paracrine fashion. On the other hand, AANAT, the rate-limiting enzyme for melatonin synthesis is expressed by cholangiocytes. Changes in melatonin secretion from cholangiocytes modulate biliary growth/loss by an autocrine loop. Abbreviations: AANAT, arylalkylamine N-acetyltransferase; GnRH, gonadotropin-releasing hormone.

found in the peripheral blood (41,43). Melatonin synthesis decreases progressively with aging as the elderly population has lower levels of melatonin (44,45). Other factors related to aging (e.g., depression, physical impairment) may contribute to the alteration of the circadian rhythm contributing to the reduction in melatonin levels in old people (44). Melatonin is mainly metabolized in the liver, where it is hydroxylated in the sixth carbon position by cytochrome P₄₅₀ mono-

oxygenases. Then, it is conjugated to sulfate and released as 6-sulfatoxymelatonin (46).

Regulation of melatonin synthesis and downstream signaling pathways following melatonin release

As stated above, melatonin synthesis in the pineal gland is modulated by light/dark information that is detected by the photosensitive ganglion cells of the retina (36). Specifically, the signal passes through the supra-chiasmatic nucleus to the pineal gland where specific “dark” and “light”-induced neural and endocrine signals co-coordinately regulate melatonin secretion. Synthesis of melatonin is inhibited by light and permitted by darkness peaking in the middle of the night in both diurnal and nocturnal animals. The function of melatonin released from the pineal gland may be also modulated by the local release of gonadotropin-releasing hormone (GnRH) from the hypothalamus (47) (Figure 1). We will discuss below some preliminary data that we have generated in our laboratory showing that chronic exposure of cholestatic rats to complete dark for one week reduces biliary proliferation and ameliorates liver function (48). GnRH also acts on the anterior pituitary to bring about the release of follicle-stimulating hormone (FSH) at low frequency GnRH pulses and luteinizing hormone (LH) at high frequency GnRH pulses. Therefore, melatonin effects may also be due to inhibition of the GnRH-mediated release of LH (49) and FSH (50), the latter also affecting biliary functions (51). Central melatonin synthesis has been also shown to be dysregulated in a number of cholestatic liver diseases. For example, abnormal melatonin circadian rhythms have been demonstrated in patients with hepatic cirrhosis and correlated with the severity of liver injury (52). Melatonin arrhythmia has been shown to be corrected following liver transplantation (53).

Melatonin exerts its effects by interacting with melatonin receptor subtypes, MT1, MT2 and MT3 [part of a G-protein coupled receptor (GPCR) family sharing a common seven-transmembrane structure] that are expressed in the gastrointestinal tract by the ileum, colon and in the liver by hepatocytes, extrahepatic biliary cells in gallbladder and cholangiocytes (54-59).

Role of melatonin on biliary growth/damage

We have recently demonstrated that melatonin inhibits cholangiocyte hyperplasia in BDL rats by interaction with

Table 1 Melatonin regulation of biliary functions

Parameter	Mechanisms	References
Inhibition of biliary hyperplasia in BDL rats	Reduced expression <i>CLOCK</i> genes by downregulation of cAMP levels, and PKA phosphorylation by interaction with MT1	(56)
Modulation of biliary hyperplasia in normal and BDL rats	<i>In vivo</i> and <i>in vitro</i> overexpression of AANAT in cholangiocytes decreased biliary proliferation	(42)
Modulation of biliary hyperplasia in ANIT-fed rats	Anti-oxidant activity	(60,61)
Inhibition of cholangiocarcinoma growth	Decreased AANAT expression and melatonin secretion leads to enhanced cholangiocarcinoma growth	(54)

Abbreviations: AANAT, arylalkylamine N-acetyltransferase; ANIT, α -naphthylisothiocyanate; BDL, bile duct ligation.

MT1 receptors (56) (Figure 1). Specifically, administration of melatonin to cholestatic BDL rats decreased ductal mass and improved serum chemistry and reduced the expression of the clock genes (*CLOCK*, *BMAL1*, *CRY1*, and *PER1* all upregulated after BDL), cAMP levels, and PKA phosphorylation in cholangiocytes (56). We propose that melatonin may be important in the management of cholestatic liver diseases.

In addition to the above described paracrine mechanism, changes in melatonin synthesis (regulated by the enzyme, AANAT, that is expressed by cholangiocytes) regulates biliary function by an autocrine mechanism (42) (Figure 1). Specifically, we have shown that AANAT expression and melatonin secretion increased in BDL and decreased in normal and BDL rats treated with AANAT Vivo-Morpholino, a treatment that decreases the biliary expression of AANAT. The decrease in AANAT expression, and subsequent lower melatonin secretion by cholangiocytes, was associated with increased biliary proliferation and increased ductal secretory activity (42) (Figure 1). Overexpression of AANAT in cholangiocytes decreased proliferative capacity of these cells (42). Local modulation of melatonin synthesis may be important for management of the balance between biliary proliferation/loss in cholangiopathies. Another study has shown that oral administration of melatonin protects from hepatotoxicity induced by α -naphthylisothiocyanate (ANIT) (60,61), a toxin that also induces biliary damage (62-64).

There is growing information regarding the role of melatonin in the regulation of the growth of cholangiocarcinoma, a devastating tumor of the biliary epithelium (54,65). In fact, melatonin has been shown to reduce cholangiocarcinoma growth and liver injury in *Opisthorchis viverrini*-infected and N-nitrosodimethylamine-treated hamsters (54,65). We have recently shown that dysregulation of the enzymatic machinery AANAT/ASMT

(N-Acetylserotonin O-methyltransferase that regulates melatonin synthesis) in cholangiocarcinoma that leads to inhibition of melatonin secretion and subsequently enhanced cholangiocarcinoma growth (54). Modulation of the AANAT/ASMT/melatonin—melatonin receptor axis may be important in the management of cholangiocarcinoma growth. Some of the effects of melatonin on biliary functions are summarized in Table 1.

Role of melatonin on liver damage

In support of the concept that melatonin protects the liver from selected pathological perturbations, a recent study has shown that this hormone protects against apoptosis during acetaminophen-induced acute liver failure (66). Another study has shown that melatonin decreases BDL-induced liver fibrosis that was evidenced by reduced levels of malondialdehyde, glutathione, luminal and lucigenin in tissue homogenates compared to BDL animals. The findings suggest that melatonin may be an effective antioxidant agent able to reduce liver fibrosis (67). Furthermore, melatonin is able to reduce dimethylnitrosamine-induced liver fibrosis in rats suggesting that this hormone may be used as a therapeutic strategy for managing liver fibrosis (68). Another study has demonstrated that melatonin inhibits nuclear factor kappa B activation and oxidative stress and protects the liver against thioacetamide-induced damage (69).

Furthermore, oral administration of melatonin (10 or 100 mg/kg body weight) has been shown to prevent the disruption of hepatic antioxidant status in cholestatic rats by its direct and indirect antioxidant action (70). Similarly, a protective role of melatonin against cholestatic oxidative stress has been demonstrated in BDL rats (71). Moreover, melatonin reduced the negative parameters of cholestasis, the degree of oxidative

Table 2 Melatonin regulation of liver damage

Parameter	Mechanisms	References
Protection against acetaminophen-induced acute liver failure	Reduction of apoptosis	(66)
Decreased BDL-induced liver fibrosis	Reduced levels of malondialdehyde, glutathione, luminal and lucigenin in tissue homogenates	(67)
Protects the liver against thioacetamide-induced damage	Inhibition of nuclear factor kappa B activation and oxidative stress	(69)
Prevention of the disruption of hepatic antioxidant status in cholestatic rats	Direct and indirect antioxidant action	(70)
Hepatoprotective effect against BDL-induced liver damage, and animal models of fibrosis and cirrhosis	Reduction of the degree of oxidative stress	(72,75-78)
Improvement of liver histology in rats with high fat diet-induced NAFLD	Attenuation of oxidative stress	(74)
Improvement of oxidative damage and rat liver mitochondrial dysfunction during hyperglycemia-induced liver injury	Attenuation of oxidative damage and rat liver mitochondrial dysfunction	(79,80)
Protective role during I/R hepatic damage	Prevention of activation of apoptosis Inhibition of IKK and JNK pathways	(86,87)

Abbreviations: BDL, bile duct ligation; NAFLD, non-alcoholic fatty liver disease; I/R, ischemia reperfusion; IKK, I κ B kinase; JNK, c-Jun NH₂-terminal kinase.

stress and provided a hepatoprotective effect against BDL-induced liver damage (72). Similarly, a protective effect of melatonin against oxidative stress induced by BDL was also described in a different study (73). Consistent with beneficial effect of melatonin on liver function, melatonin has been shown to attenuate oxidative stress, lessen liver damage, and improve liver histology in rats with high fat diet-induced non-alcoholic fatty liver disease (NAFLD), when given simultaneously with the diet (74). Recent studies have demonstrated that chronic melatonin administration protects against liver damage by attenuating oxidative stress, inflammatory responses, and apoptosis in animal models of hepatic cirrhosis and fibrosis (75-78). Melatonin has also been shown to improve oxidative damage and rat liver mitochondrial dysfunction during hyperglycemia-induced liver injury (79,80). The data suggests that melatonin may be an important nutritional supplement for diabetic patients.

Melatonin has also been shown to display a hepatoprotective effect against liver injury secondary to methanol intoxication (81). Another study has shown that liver mitochondrial damage (following acute or chronic CCl₄-induced intoxication) was improved by melatonin and cranberry flavonoids (82). Another interesting study has shown that administration of melatonin before and after irradiation reduces liver damage caused by gamma

irradiation (83). The protective effect of melatonin on liver function has also been demonstrated during ethanol administration. For example, melatonin reduces alcoholic liver injury by reducing oxidative stress, inflammatory response, and apoptosis (75). In support of these concepts, a study has demonstrated a protective role for melatonin during ischemia reperfusion (I/R) hepatic damage by maintaining preventing activation of apoptotic cell death (84,85). Another study has shown that melatonin protection against I/R occurred by inhibition of toll-like receptor signaling pathway (86). Another study has shown that melatonin protects from I/R induced injury by inhibition of I κ B kinase (IKK) and c-Jun NH₂-terminal kinase (JNK) pathways and modification of cell proliferation (87). Melatonin can also improve liver function and hepatic perfusion after hemorrhagic shock (88). The protective role of melatonin has been also observed in rats treated with CCl₄. In fact, the study showed that except for mild hydropic degeneration of the hepatocytes, a normal lobular appearance was seen in the animals treated with melatonin (89). Moreover, melatonin has been shown to play a role as potential anti-aging agent (90,91). For example, has been shown to restore hepatic mitochondrial physiology in old mice (90). The finding suggests that melatonin may be useful to reduce the deteriorative oxidative changes in mitochondria that normally occur in advanced age. Also, the hepatic mitochondrial respiratory chain activity observed

in senescence-accelerated mice was improved by melatonin treatment (91). Some of the effects of melatonin on liver damage are summarized in *Table 2*.

Summary and future perspectives

In summary, we have, following a general background on anatomical features of the biliary epithelium, discussed the vascularization of bile ducts followed by a general discussion on the secretory and proliferative response of the biliary tree with regarding to their heterogeneous profile. After a general background on melatonin synthesis, distribution and metabolism, we discuss the role of melatonin on biliary hyperplastic and neoplastic growth followed by a summary of the findings related to the protective role of melatonin against liver damage induced by a number of pathological insults. Further studies are necessary to evaluate the role of melatonin in the regulation of *CLOCK* genes and the role of *CLOCK* genes in the modulation of biliary functions.

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