Review

Melatonin, an ancient ally against pancreatic disorders

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ABSTRACT

Melatonin is mainly produced in the pineal gland of mammals with a circadian rhythm. It has also been synthesized in different organs and tissues including the gastrointestinal tract. Additionally, melatonin is widely present in plants and foodstuff. In addition to its effects on the sleep-wake cycle and reproductive regulation in photoperiodic animals, melatonin regulates a wide variety of cellular processes, participating in the control of antioxidant defenses, immune response, energy metabolism, cell growth and proliferation, having beneficial effects on most of the tissues and organs, including the pancreas.

Here, we have reviewed the recent findings related to the effects of melatonin on the physiology of the exocrine pancreas. Of major relevance, the effects of the indoleamine on pathological processes such as cancer, inflammation, diabetes, and fibrosis are also reviewed. Not less important, its effects on normal/healthy cells of the pancreas to modulate normal physiological functions are discussed.

Key words: Melatonin, cancer, inflammation, diabetes, fibrosis, pancreas, protection

1. INTRODUCTION

Melatonin, 5-methoxy-N-acetyltryptamine, is a molecule ubiquitously present in almost all species from bacteria, fungi, plants to mammals. For example, the ability of melatonin synthesis has been reported in cyanobacteria (1). In these ancient organisms, this molecule could represent an evolutionary advantage to protect them against the toxicity of oxygen (2).

In mammals, the main blood source of melatonin is generated by pinealocytes, which release melatonin into the blood stream during the dark phase of the day (3). Thus, the function of melatonin was firstly related with the control of circadian rhythms (4, 5).This process depends on light exposure, which acts as a negative regulatory signal (6). Additionally, other tissues and or organs in the body can produce significant amounts of melatonin, which include brain, retina, cochlea, Harderian gland, airway epithelium, skin, gut, liver, kidney, thyroid, pancreas, thymus, spleen, immune cells, carotid body, reproductive tract, and endothelial cells (7). Interestingly, the amount of this extrapineal indolamine is greater than the content of melatonin generated by pineal gland (7). Moreover, melatonin can also be found in several

biological fluids such as saliva, bile, cerebrospinal fluid, milk or synovial fluid (7). Last, the indole is also present in plant and a variety of foodstuff (8).

The amino acid tryptophan is the precursor for melatonin synthesis (9). This amino acid is transformed into serotonin by the enzymes of tryptophan hydrolase and tryptophan decarboxylase. Serotonin is then, metabolized by the action of the enzyme of aryalkylamine acetyltransferase (AANAT) to N-acytylserotonin which is finally converted by the N-acetylserotonin-O-methyltransferase (ASMT), formerly known as hydroxindole-O-methyltransferase (HIOMT), to form melatonin (10). The uptake of melatonin from the diet represents an additional source of this indolamine in the body (11).

Regarding its mechanisms of actions, melatonin might mediate its effects by binding to receptors (receptor-mediated effects) or via the receptor-independent activity. Cell membrane-associated receptors, and cytosolic and nuclear receptors have been described as target sites of melatonin. The classical membrane receptors, termed melatonin receptor 1 (MT1) and melatonin receptor 2 (MT2), are transmembrane structures associated with cellular membrane-G proteins (12). Binding of melatonin to these receptors recruits intracellular signaling messengers such as cyclic adenosine monophosphate (cAMP), guanosine 3',5'-cyclic monophosphate (cGMP) or calcium (Ca^{2+}) ion (10, 13–15). Other receptors comprise the cytoplasmic protein calmodulin (16) or the retinoid-related orphan receptor α (RORα) in nucleus (17). Additionally, a plethora of putative enzymes, transporters, and other proteins have been suggested as the binding sites of melatonin (18). On other hand, non-receptor mediated actions of melatonin also have been reported. These could be related to its ability to cross cell membranes. Once inside the cell, melatonin, can act as a molecule with antioxidant or pro-oxidant capacity. This dual behavior depends on the cellular context and the concentration of the indoleamine. As an antioxidant, this molecule can modulate the redox state of the cell by direct actions, detoxifying reactive oxygen species (ROS) or reactive nitrogen species (RNS), or indirectly, by modulating the activation of elements comprising of the cellular antioxidant response (19). Prooxidant actions have been observed in pathological contexts, majorly targeting mitochondria (20), as it occurs in tumor cells, and are related to the antiproliferative and cytotoxic actions observed for this indolamine in cancer cells (Figure 1) (21).

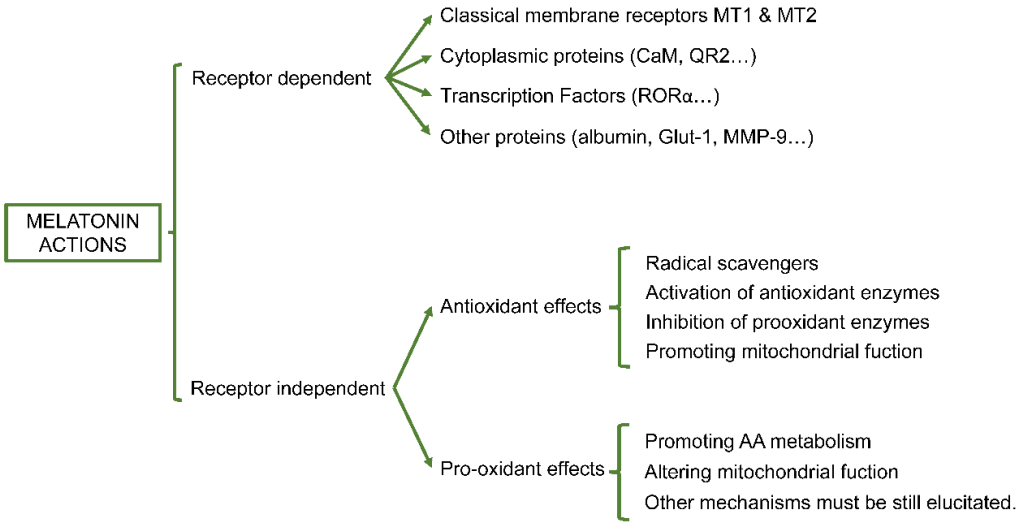

Fig. 1. Summary of the mechanisms related to the actions of melatonin.

The indoleamine can exert its effects on cellular physiology via receptor-dependent and receptor-independent mechanisms. Receptors for melatonin can be found both located at the cellular membrane (MT1 and MT2 receptors) and intracellularly (calmodulin, RORα…). Moreover, the indoleamine exhibits lipophilic property letting it cross the cellular membrane

with ease. The result will depend on the metabolic pathway linked to the route employed. In any case, it is worth noting that the effects of melatonin are cell-type and context dependent, in the sense that the indoleamine induces death of malignant cells, whereas it protects healthy/normal cells from injure.

The presence of MT1 and MT2 receptors in the pancreas has been documented (22, 23). Hence, despite it is released at the center of the brain, it indicates that melatonin can play a pivotal role in the physiology of the pancreas. In this review, we present an overview of the effects of melatonin on the physiology and pathophysiology of the pancreas. Foremost, not only are the effects of the indoleamine on abnormal cells important, but also its actions on healthy cells are relevant, adding the latter new insights onto the roles of melatonin in the body. Therefore, we will consider the effects of the indoleamine on pathological processes already set in the gland, which will be considered as part of therapeutic strategy against disease and, in addition, we will refer to the available evidence for the effects exerted on normal/healthy cells of the pancreas, which might represent a role for melatonin as peacemaker to prevent disease.

2. ANTIOXIDANT EFFECTS OF MELATONIN ON PANCREATIC OXIDATIVE STRESS INDUCED BY DIFFERENT FACTORS

Oxidative stress refers to a condition caused by the generation of free radicals that, if unresolved, it can induce damage to cellular structures and lead to diseases (24). Oxidative stress can be induced in the pancreas by different factors such as life style majorly related to alcohol consumption and/or excessive fat intake, gallstones, or smoking, among others.

Free fatty acids and amino acids can increase glucose-induced insulin secretion. In excess, these substances can lead to oxidative stress related to the development of diabetes (25). Melatonin, in addition to other hormones, can regulate insulin secretion. Therefore, the indoleamine might exert a pivotal role to prevent β-cell dysfunction, thereby, restraining the pathogenesis of diabetes (26). Evidence in this line has been provided by Su *et al*. (27), who suggested that melatonin protected insulin-secreting cells against stearic acid induced damage. Another study showed that melatonin normalized the level of free fatty acids in blood and increased insulin level to improve pancreatic function (28).

Alcohol consumption is a major cause of damage to the pancreas. Its metabolization has been related with oxidative stress within the gland (29). As such, it is considered a common cause of both acute and chronic pancreatitis (30).

Chronobiological effects of ethanol consumption suppressed melatonin production which, in turn, promotes the onset of pathological alterations in different tissues and organs (31). A study revealed that ethanol induced Ca^{2+}-mediated ROS generation in mouse pancreatic acinar cells and impaired cholecystokinin-evoked amylase secretion, thereby eliciting conditions prone to inflammation of the pancreas (32). Because chronic oxidative stress plays a key role in pathophysiology of pancreatitis it has been suggested that antioxidant supplementation could ameliorate pancreatic damage and the symptoms of the disease (33). Therefore, melatonin arises as a promising protector due to its antioxidant and anti-inflammatory properties. These findings suggest that melatonin can potentially be useful to prevent alcoholic organ damage (34). Acute pancreatitis has also been associated with gallstones. Oxidative stress together with other intracellular metabolic processes such as endoplasmic reticulum stress, defective autophagic pathway are thought to be involved in gallstones and pancreatitis (35). Additionally, occurrence of gallstones has been potentially associated with hyperinsulinemia (36). The main events of gallstones to cause the pancreas injury are the initiation and propagation of inflammation, inhibition of secretion, intracellular activation of enzymes, and the generation of inflammatory mediators (37). Because melatonin diminishes intracellular Ca^{2+} accumulation

and decreases amylase release in response to cholecystokinin, thereby avoiding putative intraglandular oxidative stress and enzyme activation (38), it could be expected a protective action by the indoleamine against putative damaging actions of gallstones to the pancreas.

Cigarette smoking is an additional risk factor of diabetes (39). A study revealed that melatonin attenuated smoking-induced hyperglycemia via preserving insulin secretion and hepatic glycogen synthesis in rats. Smoke caused alterations in the pancreas were due to ROS production and the consequent inflammation. Melatonin treatment reduced β-cell apoptosis, infiltration of CD68-cell and ROS production (40).

3. PROTECTIVE EFFECTS OF MELATONIN AGAINST PANCREATITIS

Pancreatitis is an inflammatory disease that occurs in the pancreas as a result of tissue damage induced by the release and activation of digestive enzymes in the gland itself. The secretory function of the acinar cell is highly regulated by Ca^{2+} mobilization. Therefore, alteration of this second messenger plays an important role in the pathogenesis of this inflammatory process (41). Additionally, ROS are responsible for the initiation of inflammatory disease in the pancreas (42). Interestingly, a feedback cycle between ROS generation and the impairment of Ca^{2+} signaling has been reported (43). The disturbance of enzyme secretion with a resultant intraglandular premature activation is closely related to both overproduction of ROS and abnormal Ca^{2+} signaling, and a major cause for acute pancreatitis (44). Hence, a properly controlled ROS production and Ca^{2+} mobilization is pivotal for a proper secretion of digestive enzymes and for pancreatic health maintenance. In this line, melatonin has been signaled as a putative protector of pancreatic physiology (45).

Santofimia-Castaño *et al*. (46) showed that melatonin modulated Ca^{2+} mobilization in response to overstimulation of pancreatic acinar cells with cholecystokinin. Interestingly, mobilization of Ca^{2+} by the secretagogue was attenuated in the presence of melatonin. The effect of melatonin could be explained based on a stimulated Ca^{2+} transport towards the extracellular space and by a favored reuptake into the endoplasmic reticulum. Accumulation of Ca^{2+} into mitochondria was prevented by melatonin. Not only the effect of supramaximal concentration of cholecystokinin on Ca^{2+} mobilization was regulated by melatonin, but also the oscillatory pattern evoked by physiological concentrations of the secretagogue (47).

The nuclear factor erythroid 2-related factor (Nrf2) and the antioxidant-responsive element (ARE) signaling pathways are pivotal for the antioxidant response of the cells (48). The involvement of Nrf2 in melatonin responses in pancreatic acinar cells has been observed. The indoleamine induced the activation of the transcription factor Nrf2 and the expression of Nrf2-related antioxidant enzymes via activation of non-capacitative Ca^{2+} entry. This involved phosphorylation of protein kinase C (PKC), a well-known activator of Nrf2 (49). As a consequence, the resistance of pancreatic acinar cells against ROS was increased (50).

Regarding enzyme secretion, melatonin diminished amylase release evoked by cholecystokinin. The biphasic effect of the secretagogue on enzyme secretion was not affected. However, melatonin attenuated the effect of supramaximal concentrations of cholecystokinin on amylase secretion, without significant modification of the effect exerted by physiological concentrations of the secretagogue, thereby, exerting a protective effect against overstimulation of the cells (47). Altogether, the evidence clearly indicates that melatonin modulates critical factors of pancreatic physiology, majorly Ca^{2+}-signaling, avoiding overproduction of ROS and overstimulation of enzyme secretion and, creating conditions that restrain intraglandular accumulation, and potential activation of digestive enzymes.

4. ANTITUMORAL EFFECTS OF MELATONIN IN PANCREATIC CANCER

Pancreatic cancer represents one of the most lethal oncologic pathologies with an extremely low 5-years survival rate of proximately12% in 2023 (51). Pancreatic ductal adenocarcinoma (PDAC) accounts for 85 % of pancreatic cancers and it is characterized by:

1). A high inter and intra-tumoral heterogenicity, although the tumor cells share epigenetic alterations or mutations in the proto-oncogene guanosine triphosphate-Kirsten rat sarcoma virus (GTPase KRAS) and tumor suppressor genes including tumor suppressor protein 53 (TP53), cyclin-dependent kinase inhibitor 2A (CDKN2A), and decapentaplegic homologue 4 (SMAD4) (52). Due to the PDAC heterogenicity patient stratification still represents a clinical challenge.

2). The tumor microenvironment (TME) in PDAC is characterized by a hypoxic state, high stromal deposition, nutrient deprivation, and immunosuppressive environment in which cancer cells undergo multiple resistance mechanisms allow them to survive and proliferate actively (53).

3). PDAC cells can early progress towards to a metastatic PDAC. Epithelial-to-mesenchymal transition (EMT) and targeting spreading of the tumor, mediated by soluble factors and extracellular vesicles to distant niches, are the keys in the development of metastasis (54, 55).

4). PDAC tumors exhibit wide resistance to all treatments (chemotherapy, radiotherapy and immunotherapy) (56–58). Surgical resection combined with adjuvant chemotherapy is the only curative treatment available at present, but the majority of PDAC patients are not eligible to them (59). The chemotherapeutics including gemcitabine, paclitaxel and a combination of folinic acid, 5-FU, irinotecan, and oxaliplatin (FOLFIRINOX) represent the first line choice of pharmacological treatments, although they present a high toxicity and cannot be administered to elderly patients or those with very compromised pathologies (60) (features are summarized in figure 2).

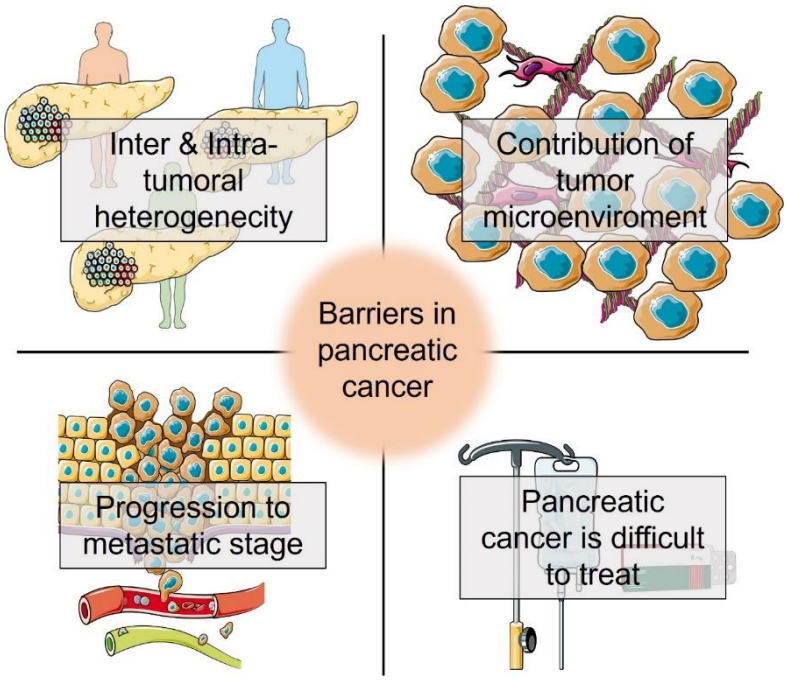

Fig. 2. Barriers in pancreatic cancer treatment.

The poor survival rate of pancreatic cancer is driven by various factors that make these types of tumors difficult to diagnose and treat. There is a wide variety of genes that are active and mutations that are developed in tumor cells. Additionally, within the same tumor, there are

different types of abnormal cells exhibiting distinct mutations and phenotypes that confer different behaviors in response to the treatments. The tumor microenvironment plays a pivotal role by providing support and protection to the tumor. Tumor cells can migrate to nearby organs and tissues, making surgical treatment difficult or impossible. Moreover, current treatments, despite increasing the life expectancy of patients who cannot undergo surgery, are still insufficient for a large number of patients. In the creation of this image some elements of Servier Medical Art were used (Creative Commons Attribution 3.0 Unported License).

One of the most interesting pharmacological properties of melatonin is its antitumoral effect. This action has been reported in a high number of oncological pathologies, including pancreatic cancer. In this part of the review, we present evidence for the antitumoral effects of melatonin in pancreatic cancer.

In Mia PaCa-2 cancer cells, melatonin at the concentrations of 1 and 2 mM reduced their viability, colony formation, migration and invasion. Induction of the c-Janus N-terminal Kinases (JNK), and activation of p44/42 mitogen-activated protein kinase (MAPK) and down-regulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) were detected after melatonin treatment in Mia PaCa-2 cells (61). This cytotoxic effect was also reported in the different cancer cell lines of Mia PaCa-2, AsPc-1, Pan-28 and gemcitabine resistant Mia PaCa-2/GR. In these cells, the indolamine inhibited NF-κB signaling pathway by suppressing phosphorylation of NF-κB inhibitor (IκBα), decreased the expression of NF-κB response genes. As a result, cell proliferation and invasion were inhibited, but enhanced gemcitabine cytotoxicity (62).

Interestingly, PANC-1 tumor cells appeared to show a high sensitivity to melatonin treatment. Melatonin promoted the pro-apoptotic activity of thse cells at the nanomolar concentrations in a receptor-dependent manner (63). Nevertheless, at this concentration the same authors did not observe statistically significant changes in cell viability (64). A reduced viability of PANC-1 cells was observed following treatment with millimolar concentrations of melatonin. The expression of mRNA for vascular endothelial growth factor was down-regulated by melatonin treatment, which was associated with a decrease in cell proliferation (65).

In the SW-1990 cell line, melatonin exhibited antiproliferative property within the millimolar range, by induction of apoptosis and the activation of necroptosis. Following melatonin treatment, the expression of B-cell lymphoma-2 protein (Bcl-2) was diminished, whereas that of bcl-2-like protein 4 (Bax) was augmented. The indoleamine also exerted a synergistic effect with gemcitabine toxicity (66).

Similar observations were found in the rat pancreatic cancer cell line of AR42J. An increase in ROS production, impairment of mitochondrial function and activation of apoptosis were induced by melatonin treatment (23, 67). The effects of melatonin seemed to involve Ca^{2+} mobilization (14).

A recent study demonstrated that cytotoxic T lymphocytes 8+ (CD8+ T cells) preincubated with melatonin prevented the drop in the expression of L-selectin (CD62L) evoked by the co-culture with Mia PaCa-2 cells. This observation indicated that melatonin might help cytotoxic T cells to infiltrate within the tumor to combat the cancer cells (68). Because of the difficulties of the defense system and chemotherapy drugs to enter deep into the tumor mass due to the existence of vast fibrotic tissue which, together with other components constitute the so-called TME (69), additional studies would be needed to elucidate the modulatory role of melatonin in the TME. The protocols that we have found in the bibliography which have been employed for the study of melatonin effects in pancreatic cancer models in the *in vitro* condition are summarized in table 1.

Table 1. Summarization of the *in vitro* studies on the effects of melatonin against pancreatic cancer.

Cell type	Actions of melatonin	Pathway/enzymes involved	Reference
Mia PaCa-2	Reduced viability, colony formation, migration, and invasion	c-Janus N-terminal Kinases (JNK), p44/42 MAPK and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)	(44)
Mia PaCa-2, AsPc-1, Pan-28 and gemcitabine resistant Mia PaCa-2/GR	Cytotoxicity	NF-κB signaling pathway	(45)
PANC-1	Pro-apoptotic, chaperone protein levels decrease	Heat shock protein 27 (HSP27) phosphorylation	(46)
PANC-1	Reduced cellular viability	Expression of vascular endothelial growth factor mRNA	(48)
SW-1990	Antiproliferative, apoptosis and necroptosis. Synergistic effect with gemcitabine	Bcl-2/Bax balance	(49)
AR42J	Antiproliferative, prooxidant, apoptosis	ROS production, impairment of mitochondria and activation of caspase-3	(23, 50)
Mia PaCa-2	Immunomodulatory	Infiltration of cytotoxic T cells	(51)

Protocols cited along the manuscript that have studied the effects and mechanisms of action of melatonin in pancreatic cancer models in vitro.

The *in vitro* effects of melatonin on pancreatic cancer have been validated in the *in vivo* studies. In the xenograft model of Mia PaCa-2 cells injected in laboratory-bred strain albino (BALB/c) nude mice, melatonin decreased the tumor mass alone or in combination with sorafenib, a multiple kinases inhibitor (70). The synergistic effect of melatonin in combination with chemotherapy has been observed in other xenograft models of nude mice injected with epithelial cells isolated from the spleen metastasis of a grade II PDAC (SW-1990) (66) and in the orthotopic pancreatic tumor-bearing nonobese diabetic/severe combined immunodeficiency disease (NOD/SCID) mice injected with AsPc-1 cells (62). In another pancreatic cancer model induced by the toxic N-nitrosobis (2-oxopropyl) amine (BOP) in *Syrian* hamsters, melatonin also has demonstrated a protective role in the oncogenic development, either alone or as an adjuvant to chemotherapy (71). The table 2 summarizes the protocols that we have found in the bibliography which have been employed for the study of melatonin effects in pancreatic cancer models in the *in vivo* condition.

Table 2. Summary of the *in vivo* studies on the effects of melatonin against pancreatic cancer.

Animal	Model of pancreatic cancer	Melatonin doses	Administration	Reference
Mouse	Xenograph with MiaPaCa cells	40 mg/kg	IP, daily	(53)
Mouse	Xenograph with SW-1990 cells	20 mg/kg	IP, daily	(49)
Mouse	Orthograph with AsPc-1 cells	25 mg/kg	IP, 4x times week	(45)
Syrian hamster	BOP-induced	2 mg/kg	Drinking water, *ad libitum*	(54)

Protocols cited along the manuscript that have studied the effects and mechanisms of action of melatonin in pancreatic cancer models in vivo.

In PDAC human patients, a lower level of melatonin was found in pancreatic tissue, which could be associated with a lower survival rate (72). Trials have been conducted in many patients with different types of cancer, in which life expectancy, quality of life or both are improved by

melatonin (73). Nevertheless, despite these findings, no human clinical trials have been performed to unravel the real effect of this indolamine as an adjuvant in the treatment of pancreatic cancer.

5. ANTIFIBROTIC EFFECTS OF MELATONIN IN THE PANCREAS

Upon injure to the pancreatic tissue, caused by different reasons such as an excessive inflammatory response, the exposure to toxic substances or tumorigenesis, fibrogenesis is activated to response for the cellular damage and preservation of the integrity of the gland (74). However, if this process losses control and perpetuates over time, an irreversible loss of pancreatic parenchyma replaced by the fiber tissues will lead to functional deficiency of the gland (75).

Within the TME, the dual roles have been described for the stroma. On the one hand, this desmoplastic reaction encapsulates the tumor and hampers the migration of tumor cells, thus blocking the development of metastases. On the other hand, this barrier can also shields the cancer cells from the immune system, radiotherapy and the availability of chemotherapy drugs. Up to date the therapies on pancreatic cancer based exclusively on the control of the stroma have not been performed clinically, even though stroma accounts for 30-80% of the tumor volume (76).

Pancreatic stellate cells (PSCs) are the major responsible cells for the development of the fibrotic tissue in the pancreas (77). In the healthy pancreas, PSCs represent a 4-7% of the total cell mass. Under physiological conditions, they are in a “non-activated state” and their function is to maintain the extracellular matrix (ECM) homeostasis. Under pathological conditions, these cells undergo a phenotypic change to a termed “activated state”, in which PSCs exhibit an increase in proliferation, migration, the ability to secrete proinflammatory cytokines and growth factors, and the deposition of ECM proteins, such as collagen or fibronectin (78).

It has been reported that melatonin inhibits the proliferation of rat and human PSCs (79, 80). Regarding the mechanisms of melatonin to reduce the PSCs viability, different targets have been found. Melatonin at the concentrations of 100 µM or 1 mM reduced the viability of PSCs. Activation of the apoptotic protein caspase-3 and decreases in the expression of cyclin A and D1 in the presence of melatonin have been reported (81). In this study, the reduced cancer cell viability caused by the highest concentration of melatonin was around 20%. JNK is a member of the MAPK family that is linked to the proliferation pathway in PSCs (82). A drop in the phosphorylation state of JNK was detected in PSCs subjected to melatonin treatment (83). The Phosphoinositide 3-kinase/Protein kinase B/mechanistic Target of Rapamycin (PI3K/Akt/mTOR) pathway regulates cell proliferation and differentiation (84). The phosphorylation state of the proteins involved in this pathway was also modulated by the indolamine to reduce proliferation of PSCs (85). Altogether, these data indicate that melatonin exhibits an anti-proliferative or cytostatic effect in this cell type.

Interestingly, PSCs do not express the classical membrane receptors of melatonin (80). The inhibition of the MT1, MT2, RORα or calmodulin did not abolish the effect of melatonin on PSCs viability (81). In these cells, melatonin appears to act as a prooxidant molecule, increasing the production of ROS and increasing the oxidation state of the cells, which was monitored as augmented level of protein carbonyls. Additionally, decreases in the total antioxidant capacity, the glutathione reduced/oxidated ratio and in the expression of superoxide dismutase (SOD) were observed upon melatonin treatment. Whereas increases in the expression of antioxidant enzymes catalytic subunit of glutamate-cysteine ligase (GCL), catalase (CAT), reduced nicotinamide adenine dinucleotide phosphate -quinone oxidoreductase 1 (NQH-1) and heme oxygenase-1 (HO-1) were detected (86). Mitochondria have also been described as the putative targets of melatonin in the exocrine pancreas, thereby,

diminishing cell viability (23). In PSCs subjected to melatonin treatment, a decrease in oxidative phosphorylation (OXPHOS) and, therefore, in the ability of the cells to obtain energy via the mitochondrial pathway was blocked by melatonin (83).

Hypoxia has been signaled as a critical component that can affect the cellular homeostatic program leading to resistance of cancer cells to treatments (87). Oxygen (O_2) is pivotal for cellular bioenergetics. Because of the low O_2 conditions occurring in the TME, in addition to the high energy demand, the cells forming the tumor mass must undergo a metabolic reprogramming that will allow them to survive (88). Hypoxia, termed as a condition in which the availability of O_2 is ≤ 1 %, is a recurrent state development in the pancreatic TME (89).

The effect of the melatonin on PSCs culture was also studied under hypoxic conditions. Hypoxia creates a prooxidant environment in which PSCs increase their activation state (82). In this context, melatonin decreased the hypoxia-induced activation reducing cell viability and diminishing the expression of the activation marker proteins α-smooth muscle actin (α-sma) and collagen (85). However, the mechanisms employed by melatonin appear to be different from those noted under normoxic conditions. Melatonin has shown interesting effects against the cellular responses that allow them to proliferate under hypoxic conditions. For example, the indoleamine suppressed the viability and angiogenesis of vascular endothelial cells subjected to hypoxia, thus exerting a potential anticancer effect. Its role as antioxidant and a free radical scavenger might be involved (90).

Because the contribution of the TME to the creation of the hypoxic microenvironment, under this condition tumor cells remain resistant to treatments, it would be of great interest if the fibrotic tissue could be switched to favor therapy against tumors. More concretely, because PSCs constitute an important part of the TME, any tool that could modulate their proliferation would represent an interesting maneuver to control the growth of the fibrotic tissue. In this line, our laboratory has performed research direct to study the effects of melatonin on PSCs physiology under hypoxia. Under hypoxic conditions, melatonin reduced lipid peroxidation and protein carbonyls oxidation evoked by hypoxia in rat PSCs. Additionally, the expression of the antioxidant enzymatic catalytic subunits of GCL, CAT, NQH-1 HO-1, SOD1, and of SOD2 were augmented. Moreover, the total antioxidant capacity was increased by melatonin treatment (91). These results could be attributed to melatonin due to an antioxidant role, rather than to the pro-oxidant action exerted under normoxic conditions.

Additional study showed that melatonin decreased the expression of cyclins A and D in PSCs subjected to hypoxia with the modulation of apoptosis by increasing the activation of caspase-3. The decreases in the expression of matrix metalloproteinases (MMP) 2, 3, 9 and 13 by melatonin were also the case, together with a slight decrease in the content of α-smooth muscle actin. All of these observations suggested that melatonin had the capacity to reduce PSCs proliferation (92).

Under the hypoxia, melatonin increased the phosphorylation of mTOR of PSCs while it was decreased in melatonin deficiency. A glycolytic shift was observed in PSCs under hypoxia, and melatonin decreased mitochondrial activity to render PSCs with lower energy supply. These activities of melatonin were related to the decreases of cell proliferation, alpha-smooth muscle actin and of collagen type 1. All of them are markers of PSCs activation (85). The dual behaviors of melatonin (antioxidant *vs* pro-oxidant) observed above depend on the cellular context in which melatonin acts on. In addition to these, an anti-inflammatory effect of melatonin under hypoxic conditions in PSCs was also observed including downregulation of NF-κB signaling, the expression of tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) cytokines genes (91).

The *in vivo* studies have confirmed the anti-fibrotic effect of melatonin in pancreatic diseases. For example, in the carbon tetrachloride (CCl_4)-induced chronic pancreatitis in mice, melatonin treatment decreased the fibrotic response, which was dependent on the inhibition of

the autophagic flux and the unfolded protein response by this indoleamine (93). Similar antifibrotic effects of melatonin were observed in rats with caerulein-induced chronic pancreatitis in which, melatonin treatment diminished lipid peroxidation and increased serum glutathione peroxidase levels (GPx) (94).

Accordingly, melatonin serum levels in chronic pancreatitis patients were lower than that found in healthy subjects. Deficiency of pancreatic brain and muscle arnt-like (Bmal1), a gene responsible for controlling the fibrogenesis was observed in PSCs. The restoration of the circadian loop with melatonin attenuated the intrapancreatic pathological changes (95). Nevertheless, no clinical trials have been initiated to date to test the anti-fibrotic effect of melatonin in pancreatitis.

6. EFFECTS OF MELATONIN IN DIABETES

Diabetes is a complex disease, in which different organs are affected. The lack, or the ineffectiveness, of insulin produced by β-cells in the Langerhans islets consequently leads to the loss of the homeostasis of glucose metabolism in type 1 (T1DM) and 2 (T2DM) diabetes mellitus, respectively (96, 97). Increased evidence highlights the beneficial effects of melatonin against T1/2DM on different β-cell deficient models (98–100). Melatonin rescued islet cells and the cell line β-TC6 from senescence induced by stearic acid. Stearic acid impaired insulin secretion due to β-cells dysfunction, which occurred via an increase in the expression of the microRNA(miR)-146a-5p or miR-8114 which repressed the V-maf musculoaponeurotic fibrosarcoma oncogene homolog A (*Mafa*). Melatonin treatment reverted the increase of these miRNAs to restore the *Mafa* expression in a T2DM model (27). Another study on T1DM showed that exposure of insulinoma cell line INS-1 to the endocrine disruptor nonylphenol altered mitochondrial activity and induced oxidative stress. Melatonin alleviated these effects, via modulation of the mouse double minute 2 homolog- suppressor protein p53- cyclin-dependent kinase inhibitor p21-Nrf2 (MDM2-p53-p21-Nrf2) axis (101). Lee *et al.* (102) reported that melatonin exhibited beneficial effects in a T1DM model, via ablation of apoptosis and senescence activation, in insulinoma-1 (INS-1) cells subjected to glucotoxicity (evoked by hyperglycemia) or lipoglucotoxicity (evoked by hyperglycemia with palmitate) exposure. The indoleamine resolved the resulting oxidative stress via an increase in the endogenous anti-oxidant defense, and improved glucose-stimulated insulin secretion.

In an *in vivo* model of T1DM induced by streptozotocin in rat, the indolamine evoked an increase in insulin secretion, which could be regarded as a consequence of islet regeneration. Melatonin-derived effect involved a reduction in NF-kB expression, which was reflected as reduced hyperglycemia and hyperlipidemia and lower oxidative stress, and the reduced levels of pro-inflammatory cytokines (IL-1β and IL-12) (103). Similarly, melatonin exerted protective effects in T2DM rat model. Treatment of diabetic rats with this indoleamine normalized the levels of serum glucose, the lipid profile and improved the insulin levels and insulin resistance compared with those observed in non-treated rats. Melatonin prevented the increase in proinflammatory cytokines and expression of proapoptotic proteins. Additionally, an increase in the antioxidant defense was detected in T2DM rats treated with melatonin (104). In an interesting study, in which a rat model of T1DM was induced by streptozotocin administration, Aasar *et al*. (105) observed that the injection of adipose tissue-derived mesenchymal stem cells pretreated with melatonin into the pancreas, promoted a regenerative and protective effect on β-cells. A decrease in the levels of the pro-inflammatory cytokine IL-17 and the proapoptotic protein caspase-3 while an increase in the levels of the anti-inflammatory cytokine IL-10 were detected.

In addition, melatonin appeared to be essential in pancreas plasticity against T2DM in pregnancy. This molecule promotes the pregnancy associated alterations of the pancreatic

morphological structures returning to its normal state. In other words, melatonin depicts major relevance in the metabolic adaptation to pregnancy and both the functionality of the β-cells and the remodeling of the pancreas during pregnancy, thus ensuring the return of the tissue to nonpregnancy conditions (106).

7. EFFECTS OF MELATONIN IN OTHER ORGANS ASSOCIATED WITH FUNCTIONS OF PACREAS

Exogenous or pineal melatonin can also influence pancreatic functions via impacts on other organs and systems of body, including the gut microbiome and hypothalamic-pituitary-adrenal (HPA) axis. Melatonin prevents gut permeability and dysbiosis, with the latter being invariably associated with a decrease in the short-chain fatty acid, butyrate (107). Butyrate is an epigenetic regulator via its capacity as a histone deacetylase inhibitor (HDACi) as well as the activation of the G-protein coupled receptors (GPR), GPR41, GPR43, GPR109A (108). Butyrate also upregulates the melatonergic pathway in cells, as shown in intestinal epithelial cells (109), with butyrate and butyrate-induced melatonin optimizing mitochondrial function and decreasing oxidant production in non-neoplastic cells, at least partly via the upregulation of sirtuin-3 (110). Butyrate and HDACi are important regulators of pancreatitis (111), fibrosis (111), pancreatic cancer (112) and T2DM (108). Consequently, some of the effects of pineal or exogenous melatonin on pancreatic disorders will be significantly influenced by concurrent effects on the gut microbiome and gut permeability (113).

As with many medical conditions, stress is an important regulator of pancreatic function, including via alterations in the HPA axis and cortisol production at the glucocorticoid receptor alpha (GR-α), including in pancreatitis (114), fibrosis (115), pancreatic cancer (116) and T2DM (117). As both melatonin (118) and butyrate (119) inhibit GR-α nuclear translocation from its cytoplasmic complex with heat shock protein (hsp)90 and p23, both melatonin and melatonin-regulated butyrate will suppress the effects of stress-associated GR-α activation in the etiology and course of pancreatic disorders. Recent work indicates that night-time processes in the dampening and resetting of body systems for the coming day may be important in the etiology of a diverse array of medical conditions, especially aging-associated conditions such as cancer and neurodegenerative disorders (120, 121). The suppression of night-time melatonin (and butyrate) may therefore, be relevant to the pathoetiology, as well as the pathophysiology, of pancreatic disorders, which will be important to determine in future research.

8. FUTURE PERSPECTIVES

Melatonin continues to be studied worldwide due to the elevated interest of the scientific community on its pleiotropic effects. Studies on the effects of the indoleamine against different types of cancer, inflammatory disease, neurodegenerative disorders, etc, have been carried out *in vitro*, using cellular models, and *in vivo*, with animals. However, the thorough researches at the clinical level in humas are currently missing, which adds major relevance to the study of melatonin.

9. CONCLUSION

The beneficial effects of melatonin on cellular physiology have been extensively studied in different tissues and organs, including the pancreas. Studies of both *in vitro* (using different types of cells) and *in vivo* (using animal models) have indicated the potentially beneficial effects of the melatonin on pancreatic physiology. Both exocrine and endocrine cells benefit from the modulatory role of melatonin on the metabolic pathways involved in damage repair,

critical for cell survival, and cell death. Of major relevance for pancreatic cancer, is the fact that melatonin exerts clear anti-inflammatory and antioxidant effects in healthy cells and prooxidant and proapoptotic effects on tumor cells. Additionally, the indoleamine depicts interesting antifibrotic mechanisms of action, which should be thoroughly considered. These features are summarized in figure 3. Moreover, the effects of melatonin seem to be cell-type and context dependent, in the sense that melatonin diminishes proliferation of abnormal or activated cells on one side but protects normal cells against physiology impairment on the other.

Finally, clinical trials in human are currently lacking. Therefore, future research is needed to unravel whether the actions already know for melatonin, obtained from *in vitro* or in animal models studies, could be applied to the treatment or prevention of human diseases.

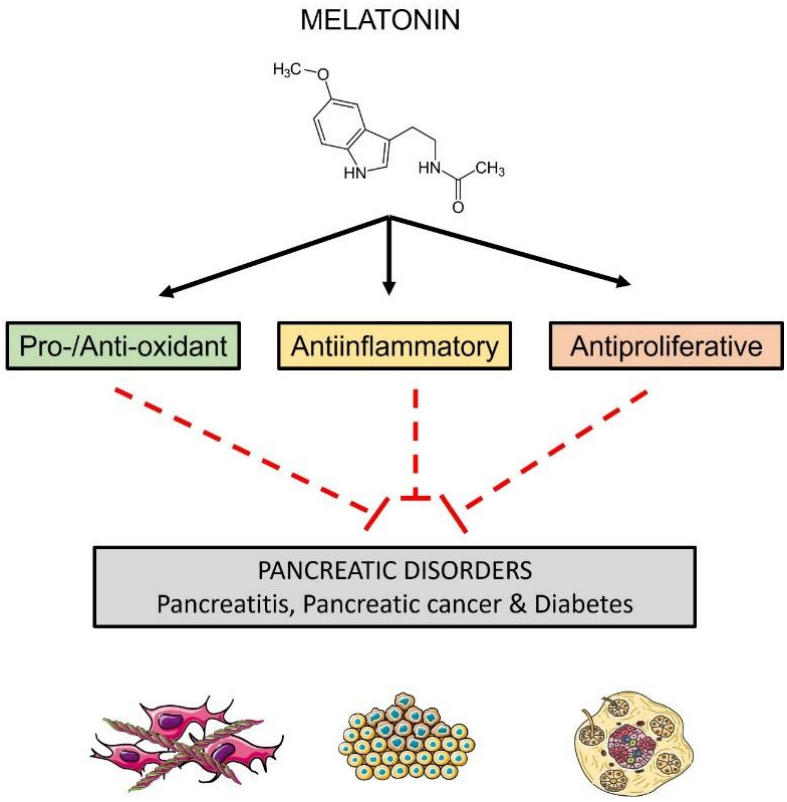

Fig. 3. Melatonin exhibits pharmacological properties that may control pancreatic disorders.

Due to its ability to control the cellular responses to oxidative stress and to modulate signaling pathways involved in cellular proliferation and inflammatory response, melatonin has been awarded a potential therapeutic role in in vitro and in vivo studies. In the creation of this image some elements of Servier Medical Art were used (Creative Commons Attribution 3.0 Unported License).

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AUTHORSHIP

M E and CO-P wrote parts or the manuscript, prepared the figures and tables, corrected and approved the final version. AG designed the manuscript, wrote parts or the manuscript, prepared the tables, corrected and approved the final version.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest. All authors contributed to the preparation of this work, and revised and approved the published version of the manuscript.

ABBREVIATIONS

Akt, Protein kinase B.
α-sma, α-smooth muscle actin.
ASMT, N-acetylserotonin-O-methyltransferase.
BALB/c, laboratory-bred strain albino mouse.
Bax, bcl-2-like protein 4.
Bcl-2, B-cell lymphoma-2 protein.
Bmal1, brain and muscle arnt-like.
Ca^{2+}, calcium.
CAT, catalase.
CCl_4, carbon tetrachloride.
GCL, glutamate-cysteine ligase.
cAMP, cyclic adenosine monophosphate.
CDKN2A, cyclin-dependent kinase inhibitor 2A.
CD8+ T cells, cytotoxic T lymphocytes 8+.
CD62L, L-selectin.
ECM, extracellular matrix.
EMT, epithelial-to-mesenchymal transition.
GPx, glutathione peroxidase levels.
GTPase KRAS, guanosine triphosphate-Kirsten rat sarcoma virus.
cGMP, guanosine 3',5'-cyclic monophosphate.
GPR, G-protein coupled receptors.
GR-α, glucocorticoid receptor Alpha.
HDACi, histone deacetylase inhibitor.
Hsp, heat shock protein.
HO-1, heme oxygenase-1.
HIOMT, hydroxindole-O-methyltransferase.
HPA, hypothalamic-pituitary-adrenal axis.
IL6, interleukin 6.
INS-1, insulinoma-1.
JNK, c-Janus N-terminal Kinases.
MAPK, mitogen-activated protein kinase.
mRNA, messenger ribonucleic acid.
miR, microRNA.
MMP, matrix metalloproteinases.
MDM2, mouse double minute 2 homolog.
mTOR, mechanistic Target of Rapamycin.
MT1, melatonin type 1 receptor.

MT2, melatonin type 2 receptor.
NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells.
NOD/SCID, nonobese diabetic/severe combined immunodeficiency disease.
NQH-1, reduced nicotinamide adenine dinucleotide phosphate -quinone oxidoreductase 1.
Nrf2, nuclear factor erythroid 2-related factor.
OXPHOS, oxidative phosphorylation.
O_2, oxygen.
PDAC, pancreatic ductal adenocarcinoma.
PI3K, phosphoinositide 3-kinase.
PKC, protein kinase C.
PSCs, pancreatic stellate cells.
p21, cyclin-dependent kinase inhibitor p21.
p53, suppressor protein p53.
ROS, reactive oxygen species.
RNS, reactive nitrogen species.
RORα, retinoid-related orphan receptor α.
SMAD4, decapentaplegic homologue 4.
SOD, superoxide dismutase.
TME, tumor microenvironment.
TP53, suppressor protein 53.
TNF-α, tumor necrosis factor alpha.
T1DM, type 1 diabetes mellitus.
T2DM, type 2 diabetes mellitus.

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