Review Article

MELATONIN: CELL DEATH MODULATOR

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ABSTRACT

Apoptosis or planned cell death is a biological phenomenon that is essential for the development and maintenance of a cell population. In this process, senescent or damaged cells are eliminated after activation of a cell death program involving participation of pro-apoptotic molecules (Fas, Fas-L, Bax, caspases 2, 3, 6, 7, 8 and 9). Molecule activation causes typical morphological changes, such as cell shrinkage, loss of adhesion to the extracellular matrix and neighboring cells, chromatin condensation, DNA fragmentation and formation of apoptotic bodies. Anti-apoptotic molecules (Bcl-2, FLIP) block the emergence and evolution of these cell changes and prevent cell death. The balance between pro- and anti-apoptotic molecules ensures tissue homeostasis. When apoptosis is out of control, it contributes to the emergence of several neoplastic, autoimmune and neurodegenerative diseases. Several inducing and inhibiting agents of apoptosis are recognized as potential weapons in the fight against disorders related to cell proliferation and death among, among which stand hormones out. Melatonin has been reported as an important anti-apoptotic agent in various tissues by reducing cell calcium uptake, mitigating the production of oxygen reactive species and decreasing pro-apoptotic proteins, such as Bax. The knowledge of new agents capable of acting on the course of apoptosis is important and valuable for developing further therapies against various diseases. Thus, the objective of this review was to clarify the main aspects of cell death by apoptosis and the role of melatonin in this process

Key Words: Melatonin. Pineal Gland. Apoptosis. Caspases. Apoptosis Regulatory Proteins.

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Introduction

The biological development and cell turnover of a tissue are regulated by a planned death process called apoptosis, which is responsible for the elimination of senescing or undesirable cells. This phenomenon was first described by Kerr et al. (1972)[1]. It is essential in the proliferation, differentiation, and survival of cells in processes of organogenesis, hematopoiesis, tissue replacement, organ atrophy and metamorphosis, inflammatory response and secretion of cells after damage by genotoxic agents[2,3].

In the process of apoptosis, cell death is induced by the activation of a genetically and biochemically regulated cell death system, involving the participation of pro-apoptotic molecules (Fas, Fas-L, Bax, Caspases 2, 3, 6, 7, 8 and 9) which are able to cause drastic morphological and functional alterations. On the other hand, this process could be inhibited by the activation of anti-apoptotic molecules (Bcl-2, FLIP) that block the appearance and evolution of these cell alterations. Thus, homeostasis (essential structural and functional balance for the survival of a cell population) depends on the balance between the activation of pro- and anti-apoptotic molecules[4,5].

When unregulated, apoptosis could contribute to the appearance of various neoplastic, autoimmune and neurodegenerative diseases[2,5,6].

Several apoptosis inducing and inhibiting agents are recognized as potential weapons in the fight against diseases related to cell proliferation and cell death disorders. Among these agents, steroid and non-steroid hormones are particularly noteworthy. Steroid hormones could present both pro-apoptotic[7] and anti-apoptotic[8] activity.

Melatonin, a non-steroid hormone (indolamine) produced in the pineal gland. It influences the regulation of the neuroendocrine

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system, the control of circadian rhythms for various physiological processes, and the anti-apoptotic system in various cell death-induced vertebrate tissues[9-12]. However, the mechanism through which melatonin exerts its inhibitory effect on cell death remains unclear. This review initially summarizes the apoptotic process and pathways and the anti-apoptotic activity of melatonin through the inhibition of the apoptotic pathways and activation of the survival pathways.

Apoptosis

The integrity and homeostasis of multicellular organisms requires various cellular and molecular mechanisms to function perfectly, such as the one engaged in planned cell death. In this process, cell population is rigorously controlled by genetic and biochemical factors, which are in charge of the activation of specific anti-apoptotic molecules[13]. The activation of these molecules that signal cell death causes typical morphological and ultrastructural alterations in the apoptosis process: cell retraction, loss of adhesion to the extracellular matrix and neighboring cells, chromatin condensation, DNA internucleosomal fragmentation and the formation of cytoplasmic blebs referred to as apoptotic bodies[3,14]. In this process, the plasma membrane remains whole, but undergoes structural alterations, such as the distribution of phosphatidylserine on the outer layer of the membrane, which is a signal for phagocyte recognition[15]. In this manner, the dead cell is quickly removed without leakage of cytoplasmic content, thus avoiding an intense inflammatory response[6].

Generally, the inflammatory response is present at necrosis, a type of cell death which is morphologically and biochemically different from apoptosis, in which the injury to the cell causes increased volume, chromatin aggregation, cytoplasmic disorganization and loss of plasma membrane integrity. Consequently, there is leakage of cytoplasmic content, thus causing damage to neighboring cells and local inflammatory reaction[3,6].

Apoptosis inducers and inhibitors

Various factors are able to induce the apoptosis process through the activation of pro-apoptotic molecules. Among these are DNA damage (caused by ionizing radiation or chemotherapic agents), growth factors and nutrient deprivation, thermal shock and intracellular buildup of toxic oxygen reactives[16].

Apoptosis inhibitors modulate the activation of anti-apoptotic molecules. Among them, we underscore the factors of extracellular matrix, zinc, some aminoacids and steroid and non-steroid hormones[16].

Caspases: cell death-effector molecules

The morphological alterations observed in cells undergoing apoptosis are the final result of the activation of enzymes referred to as caspases. These enzymes modulate the apoptotic process and serve as primary markers in apoptosis tests even before the morphological signs become evident. These enzymes are responsible for the cleavage of substrates that contain aspartic acid residue, such as the poly (ADP ribose) polymerase, enzymes, cell cycle regulating proteins, structural proteins, such as laminin and actin, among others[13,17]. In humans, more than 14 types of caspases are recognized, but part of them have pro-apoptotic properties[13]. Caspases-1, 4 and 5, for example, are recognized as being important in the inflammatory process, whereas caspases-2, 3 and 10 are mostly (if not exclusively) involved in apoptosis[13].

Depending on the event in which they participate in the apoptosis process, caspases can be referred to as initiators (caspases 8 and 10) or executors (caspases 3 and 7). Genetic studies show that apoptosis, just like any other metabolic process, can be interrupted by mutation[2]. In mice, for example, the deletion or mutation of the gene that encodes the Mrad1 protein, which plays a vital role in DNA repair and cell cycle control, results in the abolition of the planned cell death process, with the appearance of skin tumors[18].

Apoptosis activation pathways

Apoptosis can be initiated by external stimuli through the activation of specific receptors present on the cell surface (**extrinsic** pathway or cell death receptor pathway), referred to as death receptors, or by intracellular stress (**intrinsic** or mitochondrial pathway). Both pathways culminate with the activation of caspases that lead to cell death[19,20].

The extrinsic pathway is responsible for the elimination of undesirable cells during the development, maturation of the immune system and immune mediated tumor destruction (immune vigilance)[17]. This pathway is triggered by the interaction of specific ligands to a group of cognate receptors belonging to the family of the tumor necrosis factor (TNF), capable of causing the activation of caspase-3 and, consequently, cell death[3,17].

The receptor Fas (CD95/APO-1), a 45 kDa cell surface molecule belonging to the TNF family, has become a paradigm for studies on extrinsic pathway of apoptosis. Its ligand (Fas-L/CD178) is a 37 kDa protein belonging to the superfamily of the TNFs [20-23]. Fas and Fas-L are engaged in the cell death process; after the interaction of Fas-L on the cell surface with the Fas receptor, there is formation of aggregates in the form of trimers, which bond to adaptor protein FADD *(Fas associated death domain)* present in the cytoplasm. This molecular complex bonds to pro-caspase-8, resulting in the formation of the DISC *(Death Inducing Signalling Complex)* complex, where the activation (dimerization/cleavage) of pro-caspase-8 occurs, thus resulting in the activation of the effector caspase-3, which culminates in cell death[17].

The intrinsic pathway is activated by intracellular or extracellular stress, such as growth factor deprivation, DNA damage, hypoxia, and others. In response to these factors, the mitochondria undergoes changes in its inner membrane potential, in terms of membrane permeability and increase of matrix density. Additionally, some authors report that these organelles assume perinuclear distribution during this process[24]. These mitochondrial alterations can be crucial for the triggering of death, and could facilitate the translocation of mitochondrial proteins, blockage of ATP synthesis and increase in the production of reactive oxygen species, which leads to the oxidation of lipids, proteins and nucleic acids, in addition to contributing to the activation of caspases-9 and 3. Mitochondrial derangement could also facilitate the release of cytochrome C to the cytoplasm, where it forms a complex with the activation factor associated with apoptosis-1 (APAF-1) and caspase-9, referred to as apoptosome, which promotes the cleavage of pro-caspase-9, thus releasing

active caspase-9, which is capable of activating caspase-3 and provoking apoptosis[3,16,17].

It should be mentioned that, in both pathways, the activation of caspase-3 is the crucial, irreversible point of the cell death phenomenon; i.e., at this stage there are no mechanisms capable of reversing apoptosis[16].

Inhibitors of caspase activation in apoptosis

The recruitment and activation of pro-caspase-8 are regulated by molecules such as the FLIP *(FLICE-like inhibitory protein)*, homolog of caspase-8 with important differences, such as the lack of catalytic residue, which renders it incapable of performing proteolytic activity.

The literature has not reached a consensus regarding the pro- or anti-apoptotic properties of FLIP. Studies show that, when present at low levels (in normal cells), FLIP increases the activation of caspase-8; however, when there are high levels of the protein in tumor cells, it competes with caspase-8 for the bond site of the Fas-FADD complex, thus inhibiting apoptosis[17,19].

It should be noted that some proteins in the Bcl-2 family also modulate the activation of caspases, whether inhibiting the activation of these proteases, such as Bcl-2, Bcl-xl, Bcl-w, or promoting apoptosis, such as Bax, Bax, Bid, Bak and Bcl-xs [5, 24-27].

The Bcl-2 protein, for example, inhibits the release of cytochrome C by the mitochondrias, thus preventing cell death[11,12,25]. However, although the antiapoptotic role of these proteins has been thoroughly documented, little is known about the precise mechanism through which Bcl-2 and the other members of this family can act in this process[4].

Melatonin

Melatonin (N-acetyl-5-metoxytryptamine) has already been used in medical practice for many years, being safe and well-tolerated, even at high doses, thus easily crossing the blood-brain barrier[27]. In addition to being used to increase sleep efficiency[28], treat jet lag, improve the cardiovascular system[29], as an anti-aging drug[28], diet supplement, or as protection against the appearance of tumors[30], there is evidence that it could play a part in controlling the cell death process, acting in apoptosis[11,12,31, 32].

Melatonin action in apoptosis

Several studies show that melatonin plays a part both in the extrinsic pathway, by modulating the expression of death receptors, and in the intrinsic pathway, by eliminating from the cytoplasm the oxidating free radicals that can be generated by the mitochondria.

Some authors show an anti-apoptotic action of melatonin in different organs, such as thymus, kidney, brain and liver, attributing this mainly to its antioxidating properties[7-12,33,34] when eliminating radicals hydroxyl (OH^-), peroxyl (ROO^-) 26, superoxides[4] and cardiolipin oxidation from mitochondrias[35]. In the lacrimal glands of hamsters, this hormone prevents cell damage caused by the buildup of porphyrins, due to its capacity of decreasing the RNAm synthesis of aminolevulinate synthetase, enzyme involved in the production of porphyrins, and also due to increasing the levels of RNAm of antioxidating enzymes such as manganese superoxide dismutase (Mn-SOD) and copper-zinc superoxide dismutase (Cu-Zn-SOD)[36]. The synthesis of glutathione peroxidase, another very important enzyme for the elimination of free radicals from the organism, also increases in the brains of mice treated with melatonin[27], thus proving that this hormone also acts over other enzymes that provide protection against toxic reactives. Melatonin is also capable of acting as an antioxidant when negatively regulating the levels of nitric oxide synthase, involved in the synthesis of nitric oxide, as observed in mice submitted to ischemic brain injury and treated with the hormone[33].

Some authors have reported that, as the melatonin levels decrease with aging, there would be an inhibition of the SIRTI gene that regulates circadian rhythm, thus leading to its derangement, with apoptosis inhibition and increase in the susceptibility to tumors[37].

In another study involving the central nervous system (CNS), Lima et al.[38] have observed that the administration of melatonin to pinealectomized mice induced to epilepsy with pilocarpine caused a reduction in the number of TUNEL-positive cells in various limbic areas of the CNS. This indicates a neuroprotector effect of this hormone during the epileptic state, suggesting that it can be used as adjuvant in anticonvulsive therapy. Wang[39] has reported that the melatonin action acts on the prevention of neurodegenerative diseases by inhibiting the intrinsic pathway of apoptosis and activating the survival pathways.

The modulation of immune elements is also a mechanism through which melatonin can inhibit apoptosis, thus inducing the release of cytokines such as interleukin (IL)-4. Some authors have shown that melatonin is capable of avoiding cell death of hematopoietic progenitors after chemotherapy, both in vivo and in vitro, and that this effect is mediated by Th2-type T-cells, which are stimulated by this hormone to release IL-4, which induces the activation of stromal cells[40]. These cells, in turn, begin to release granulocyte macrophage colony-stimulating factor (GM-CSF), thus increasing the number of granulocyte macrophage (GM-CFU) colony forming units. However, it is important to observe that this effect takes place only with precursors of the GM-CFU lineage. Other studies have shown that melatonin is not capable of influencing tumor growth and the appearance of methastases[41]. However, when used as adjuvant in the treatment of cancer, it obtains good results in controlling the disease[42].

Melatonin can also interact with nuclear receptors, exerting direct genomic action, altering the expression of apoptosis genes and thus inhibiting cell death.

In culture, melatonin inhibits the proliferation of human breast cancer cells (MCF-7), inducing a cell cycle arrest depending on an increase of protein p21WAF1 expression, which is mediated by pathway p53, thus disrupting the balance between mitosis and apoptosis[43]. On the other hand, in thymocytes treated with dexamethasone, it has been observed that the administration of melatonin was able to reverse the apoptotic process caused by the glycocorticoid, reducing DNA fragmentation and Bax (pro-apoptotic protein of the Bax RNAm) levels[10]. In that sense, studies have shown that melatonin is capable of reducing the activity in caspases-9 and 3 induced buy the increased concentration of cytoplasmic calcium in human leukocytes, due to the activation of Bax with release of cytochrome C, thus leading to reduced apoptotic activity[44].

As previously mentioned, the anti-apoptotic action of

melatonin has also been reported in nervous and renal tissues[7]; in this case, treatment with the hormone causes a decrease in the expression levels of proteins Fas, Fas-L and p-53, along with an increase in the expression of Bcl-2[11]. However, the mechanisms involved in the control of these genes' expression, as well as the end of the apoptosis cascade (caspase-3-cleaved) require further investigation.

Considering the importance of the phenomenon of apoptosis in various proliferative affections, such as neoplasia, and other evidence of the melatonin action in the apoptotic process, this information is important to the formulation of future therapies for the clinical practice.

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