Review

Melatonin: Protection of the intervertebral disc

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ABSTRACT

Low back pain (lumbar pain) due to injury of or damage to intervertebral discs is common in all societies. The loss of work time as a result of this problem is massive. Recent research suggests that melatonin may prevent or counteract intervertebral disc damage. This may be especially relevant in aging populations given that endogenous melatonin, in most individuals, dwindles with increasing age. The publications related to melatonin and its protection of the intervertebral disc are reviewed herein, including definition of some molecular mechanisms that account for melatonin's protective actions.

Key words: Melatonin, collagen I, collagen II, annulus fibrosis, nucleus pulposus, endochondral endplates, bone health, chondrocyte.

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1. INTRODUCTION

Several years ago, a review article was published in which the use of melatonin was proposed as a possible treatment to reduce pathological connective tissue growth commonly known as fibrosis (1). These conditions involve unusual accumulations of both collagen and extracellular matrix. Subsequently, several reports have focused interest on the role of melatonin in maintaining the integrity of the collagenous cushions between the spinal vertebrae. These findings are noteworthy because, when these cushions are damaged, the consequence is often debilitating back pain. In particular, low back pain is a very common complaint of adults in all age groups and it has a major impact on societies world-wide and on the economy.

2. INTERVERTEBRAL DISC

The vertebral column is the essential support for the remainder of the skeleton and for maintaining body posture. The bony portion of the column is constructed of individual calcified vertebrae separated from each other by 25 flexible collagenous cushions referred to as intervertebral discs (IVD). Each disc consists of two components that include the annulus fibrosis (AF) and the nucleus pulposus (NP) along with the cartilaginous endplates of the

vertebrae. The AF is primarily a collagenous bundle that wraps around the more fluid NP between two successive vertebrae keeping the NP in place (Figure. 1). The NP is in the center of the IVD and is a flexible gel-like material. The cartilaginous endplates are thin layers of hyaline cartilage that separate IVD from the vertebrae.

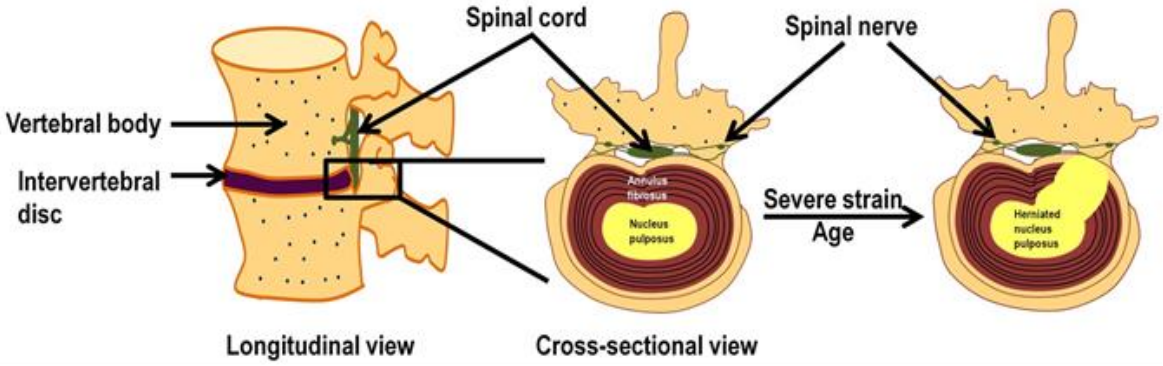

Fig. 1. Longitudinal and cross-sectional views of the vertebrae with an intervertebral disc (IVD), which intervenes between adjacent vertebrae.

The IVD consists of a strong outer ring consisting of primarily type I collagen, the annulus fibrosis (AF), and an inner more fluid nucleus pulposus (NP), which contains type II collagen and associated extracellular matrix components, e.g., glycosaminoglycans, etc. In the event of a genetic defect or a result of increasing age or severe strain on the vertebral column, the AF may rupture leading to the herniation of the NP. In this illustration, the herniated tissue is shown compressing the spinal nerve which would lead to pain, regional numbness and, in extreme cases, muscle atrophy.

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The NP has a high-water content, type II collagen, cells that resemble chondroblasts/cytes and an extracellular matrix (ECM) of sulfated glycosaminoglycans. The AF and NP provide stability and flexibility to the vertebral column with the AF being stretchable while also resisting compression and preventing the bony vertebrae from coming in contact. The gelatinous NP has a consistency which allows it to respond to mechanical stress and direct it to the AF and the vertebral endplates.

The health of the spinal column requires that the NP be maintained in the center of IVD. Under severe mechanical stress the NP pressure exerted on the NP can cause the rupture of and herniate through the AF. This is most common in the discs of the cervical and lumbar regions. Such herniations can be painful and seriously requiring repair; such herniations can lead to numbness and weakness of the muscles of the extremities when it compresses spinal nerves (Figure 1). The extrusion of the NP also elicits an inflammatory reaction that contributes to the pain and damages the surrounding tissue including nerves and bone. The estimated economic loss to industry due to “back problems” is in the billions of dollars annually.

3. INTERVERTEBRAL DISC: PROTECTION BY MELATONIN

The first reports claiming that exogenous melatonin administration is an aid in the repair of experimentally-damaged IVD were published in 2006 (2, 3). Of specific interest in the first study was the role of melatonin in regulating the expression of transforming growth factor-beta 1 (TGF-β1), a critical multifunctional cytokine that has reparative actions in collagen-rich tissues such as of the IVD. In rats in which the IVD was damaged by making a cut in the AF (4), the immunoreactive TGF-β1 and the trabecular morphology of the adjacent vertebrae were evaluated. Damage to the IVD induced collagen fiber disorganization, thinning of the vertebral bony trabeculae and an increase in immunoreactive TGF-β1 in the chondrocytes of the AF. Following subcutaneously injected melatonin, the damaged discs exhibited improved organization of the collagen fibers, thickening of the vertebral bony trabeculae and especially a very marked rise in immunoreactive TGF-β1 expression in chondrocytes. The large melatonin-induced rise in TGF-β1 is consistent with its reparative actions (5). Also, the restoration of the width of the trabeculae is in line with what is currently known about melatonin's ability to promote bone formation in osteoporotic tissue (6). The authors suggested that in addition to stimulating TGF-β1, melatonin assisted in the repair of the IVD by reducing inflammation and due to its ability to limit oxidative stress (2).

In a related study directed to determining the effects of melatonin in reducing bone loss in ovariectomized rats, Oktem and colleagues (3) observed that inducible nitric oxide synthase (iNOS) exhibited increased expression along with a rise in the number of apoptotic cells in the NP and epiphyseal cartilage, effects that were reversed in the ovariectomized animals treated with melatonin. iNOS was estimated since an increase in this enzyme is critically related to the pathogenics of osteoporosis.

Following the early reports (2, 3, 7), a long interval elapsed before interest in the function of melatonin at the IVD was revived. While the causes of IVD degeneration and the resulting back pain are multifactorial, they are often a result of a spontaneous abnormal rise in NP cell proliferation (8). The group of Li *et al* (9) have had a long investigative history of the molecular processes related to the health of the IVD. To approach the role of melatonin, they first documented that the membrane receptors for melatonin (MT1 and MT2) are present in the human IVD including on cells of the NP (10). In cultured human NP cells collected from lumbar IVD obtained from patients undergoing discectomy, melatonin significantly reduced their proliferation with the response being dose-dependent (10). Associated with this change, melatonin also restrained gene expression for cyclin D1, matrix metalloproteinase-3 and -9, which degrade the ECM, and proliferating cell nuclear antigen (PCNA) in NP cells while upregulating collagen II alpha 1 chain (COL2A1), proteoglycans and aggrecans in NP cells. These findings indicate that melatonin preserved the ECM of the NP. This is important since degradation of the ECM is indicative of IVD degeneration. All of the observed changes were obviated by treatment of the cells with a non-selective MT1/MT2 blocker, luzindole, indicating the actions of melatonin on NP cells were membrane receptor-mediated. The results are of special interest since degradation of the IVD is generally associated with aging, a time at which circulating melatonin concentrations are dropping; thus, maintaining higher melatonin levels into advanced age may also assist in postponing degenerative changes in the IVD. The results reported by Li *et al* (10) are also consistent with publications claiming that pinealectomy, which greatly attenuates levels of melatonin in the blood, causes premature loss of spinal column integrity leading to scoliosis (7).

The findings related to the role of melatonin in preserving the health of the IVD were extended by Zhang *et al* (11) using additional endpoints. The authors used endplate chondrocytes (EPC) isolated from damaged IVD (by needle penetration) of rats. Elevated apoptosis and calcification of EPC are associated with IVD degeneration. When incubated with melatonin, EPC exhibited a reduced apoptotic cell death and less calcification. Melatonin treatment also upregulated SIRT1 activity in EPC which promoted autophagy; SIRT1 inhibition (with EX527) counteracted the actions of melatonin in terms of apoptosis and EPC calcification. In an *in vivo* study, IVD damage was likewise attenuated by melatonin. Moreover, melatonin preserved ECM integrity in the NP. Whether melatonin receptors were involved in these responses was not tested. Clearly, melatonin targets both the EPC and NP to maintain the strength of the IVD. Optimal health of the cartilaginous endplates are important in preserving the integrity of the IVD (see Figure 2 for details).

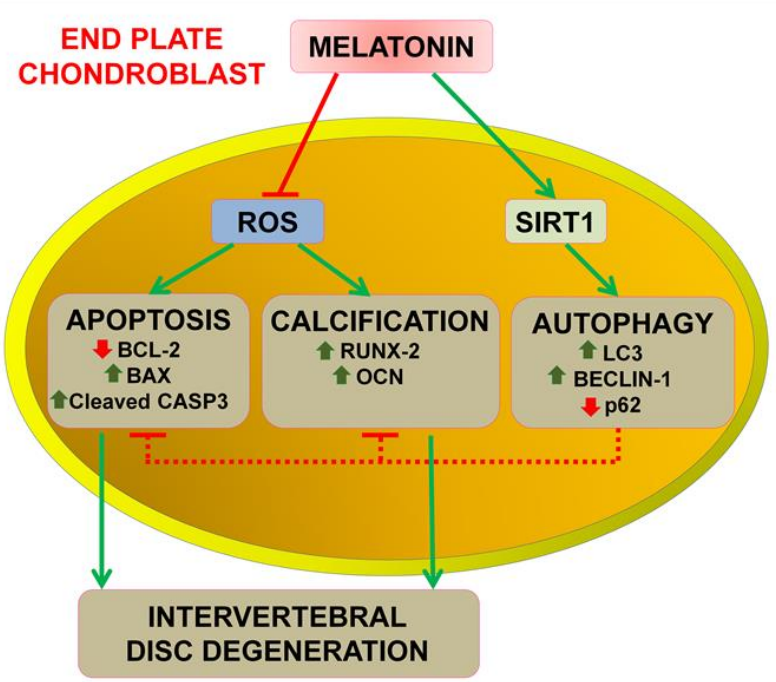

Fig. 2. Potential mechanisms by which melatonin reportedly reduces intervertebral disc (IVD) damage or degeneration.

Generation of pathological levels of reactive oxygen species (ROS) induces apoptosis and calcification of vertebral chondrocytes, which are cellular elements of the cartilaginous endplate (CEP). The CEP is a layer of hydrated tissue that lies between each IVD and the adjacent vertebrae. The health of the CEP is important in the maintenance of the IVD since it aids in nutrient transfer and waste removal from the IVD, an avascular structure. Melatonin stimulates SIRT1 which enhances autophagy of endplate chondrocytes causing modulation of apoptosis and calcification by inhibiting these processes, and averting IVD degradation. Also, melatonin may limit apoptosis and calcification of endplate chondrocytes by directly or indirectly scavenging ROS, contributing to the reduction of IVD degeneration. IVD consists of the annulus fibrosis (AF) and nucleus pulposus (NP). OCN = osteocalcin; LC-3 = microtubule-associated protein 1A/1B-light chain 3.

Two studies considered the roles of oxidative stress and mitochondrial dysfunction as they relate to IVD injury (12, 13). Melatonin was tested in these experiments since it is a well-known inhibitor of oxidative stress (14-16) and is important in maintaining optimal mitochondrial function (17-20). As anticipated, melatonin blocked tert-butyl-hydroperoxide (*t*-BHP) induced NP cell apoptosis; *t*-BHP is a free radical generating agent. Mitophagy and Parkin were upregulated by melatonin when mitophagy was suppressed using cyclosporine A, the ability of melatonin to influence this process was, in part, negated. Also, consistent with the findings summarized in the previous paragraphs, melatonin maintained collagen II, aggrecans, and SOX-9 (transcription factor SOX-9) levels of the ECM while attenuating the enzymatic activities of matrix metalloproteinase-13 (MMP-13) and ADAMT-5 (a disintegrin and metalloproteinase with thrombospondin motif 5), both of which normally degrade the ECM.

Chen *et al* (11) point out that the effects of melatonin are of special importance since the IVD is avascular; thus, melatonin can only protect this structure from degradation if it is capable of permeating into it. This contrasts with other chemicals, which are effective in protecting isolated NP and EPC from damage but are not useful in vivo since they are incapable of penetrating the IVD.

When isolated rat NP cells were challenged with H_2O_2, a strong oxidizing agent, it proved to be cytotoxic with an elevated rate of apoptosis and higher reactive oxygen species and malondialdehyde (MDA) levels, a product of lipid peroxidation (12, 21). In addition to reducing each of these parameters, melatonin upregulated antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH) and elevated collagen II and aggrecans production by NP cells. After H_2O_2 exposure, Bcl2 declined, Bax increased, cytochrome c release was increased associated with a reduction in mitochondrial cytochrome c levels. Cleaved caspase 3 and caspase 9 were likewise increased as a consequence of incubation of NP cells with H_2O_2 consistent with the high rate of apoptosis. As proof of melatonin's anti-apoptotic actions in NP cells, their treatment with the indole eliminated all of the molecular changes that accompanied H_2O_2 exposure.

4. ADDITIONAL SUPPORTIVE DATA

Just as this report was nearing completion, a publication appeared the results of which are consistent with the data summarized herein. Pintor (22) observed that human articular chondrocytes (obtained from cartilage of the knee joint) accelerated their production of extracellular matrix (proteoglycans) when exposed to melatonin. Also based on western blot analysis, melatonin enhanced collagen II by the incubated chondrocytes. By incubating the cells with the MT1/MT2 melatonin membrane receptor blocker (luzindole) or with HD-92, a specific blocker of the MT2 receptor, the author showed that the stimulatory actions of melatonin were mediated via the MT2 receptor. The findings have relevance to the chondrocytes and extracellular matrix of the cartilaginous endplates of the intervertebral discs since they are continually renewed (23) and repaired if damaged, e.g., in conditions such as osteoarthritis (24, 25). As noted above, healthy vertebral endochondral endplates are important in maintaining an optimally functional IVD.

Damaged cartilage often produces abnormal extracellular matrix which leads to pain and interferes with normal reparative processes. Under these conditions, autologous chondrocyte transplantation is often used as a corrective measure (26). The collective findings summarized

herein indicate that exposing the cells to be transplanted to melatonin or giving individuals who receive such transplants supplemental melatonin for a period of time may improve the success rate in the cartilaginous endplates of the intervertebral discs which are continually renewed and, when damaged, repaired (26, 27). Melatonin also enhances chondrogenic cell differentiation from human mesenchymal stem cells (28) which makes them more readily available for autologous transplantation.

Of special interest are the just-published findings of Fu *et al* (29) who showed the chondrocytes (derived from the ribcages of embryonic mice), like perhaps all other cells (30, 31) are capable of producing melatonin. This proposal was based on the presence of mRNA expression of the genes which encode the two enzymes, i.e., arylalkylamine N-acetyltransferase (AANAT) and hydroxyindole-O-methyltransferase (HIOMT) (also known as acetylserotonin-methyltransferase (ASMT) that convert serotonin to melatonin. They also reported that melatonin regulates cartilage growth and maturation. While this study did not specifically examine IVD-derived chondrocytes, it seems likely that they also produce melatonin.

5. CONCLUDING REMARKS

The capacity of melatonin to protect the IVD from experimental degenerative changes has important clinical implications. The pain associated with IVD degradation is a major cause of disability and severely compromises life quality. The socioeconomic losses and high health costs associated with this condition are serious public health concerns. Considering that vertebral column injury is often related to aging and endogenously-produced circulating melatonin levels generally drop with age (32), melatonin supplementation in late life may counter the frequency or severity of this debilitating condition, as well as reduce the associated pain (33). The protective actions of melatonin likely stem from its ability to sustain the IVD but also to improve trabecular bone formation in the vertebrae (8, 34).

If the experimental findings described herein in terms of the protective actions of melatonin against intervertebral disc damage is verified in the human, it would obviously be highly significant. Hopefully, clinical trials will be initiated soon to test the potential role of melatonin in maintaining the integrity of not only the chondrogenic endplates of the vertebral disc, but other cartilaginous sites as well and including the associated bony tissue (6).

Finally, the fact that melatonin inhibits pathological fibrosis by reducing excessive collagen formation (1) while it promotes collagen synthesis in the IVD is another example of the dichotomous actions of this functionally-diverse molecule. This context specificity is also apparent in other cells and other functions (35).

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AUTHORSHIP

RJR, SARC and RS participated in writing the article and preparing the figures. All co-authors read and approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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