Melatonin: A hypothesis regarding its use to treat Wilson disease

Ramaswamy Sharma⁎, Russel J. Reiter, Qiang Ma

Department of Cell Systems and Anatomy, UT Health San Antonio, San Antonio, TX 78229, USA

ARTICLE INFO

Keywords:
Hepatolenticular degeneration
Liver toxicity
Hepatic Fibrosis
Copper toxicity
Radical scavenging
Anti-fibrotic
ER stress
Metal chelation

ABSTRACT

Wilson disease is associated with excessive copper accumulation in cells, primarily in the liver and brain. The subcellular lesions caused by an excess of this essential metal accounts for many of the signs of Wilson disease. The drugs used to treat this disease are not always effective, and depending on dose, they may have collateral toxicity. Melatonin is an endogenously-produced molecule that functions as a copper chelator, a potent antioxidant, and as a suppressor of endoplasmic reticulum stress and the unfolded protein response in both the liver and the brain, while also reducing fibrosis/cirrhosis in the liver. Melatonin is inexpensive, non-toxic and can be administered via any route. Melatonin should be tested for its potential utility in experimental models of Wilson disease with extension to the human if melatonin proves to be effective in the animal studies.

Introduction

Wilson disease (WD), also known as hepatolenticular degeneration, is a genetic condition that results in the excessive accumulation of copper in many cells, particularly causing damage to hepatocytes and neural tissue [1]. While copper is an essential trace element, functioning as a cofactor of enzymes such as mitochondrial cytochrome c oxidase and superoxide dismutase, (SOD1), it becomes toxic when it accumulates excessively in cells (copper toxicosis). The clinical signs of this disease resemble those of several other conditions, making an early or definitive diagnosis sometimes difficult. The major clinical signs of WD relate to hepatic damage which can lead to liver cirrhosis, Kaysers-Fleischer rings which encircle the iris of the eyes (resulting from excess copper deposition), acute episodes of hemolysis, involvement of the nervous system leading to neuropsychiatric problems, and eventually hepatic failure, a life-threatening condition. Identifying WD in children and in adults with unrelated liver disease may be challenging.

In addition to the clinical signs, to aid in the diagnosis, a number of laboratory measurements including copper levels in liver, serum, and bile and serum ceruloplasmin are evaluated. These measurements are used to calculate the Leipzig score with a score of > 4 indicating the high likelihood of WD [2]. Also, genetic tests are available to aid diagnosis.

Pathology of Wilson disease

WD is an autosomal-recessive condition resulting from a defect in the gene (ATP7B) that encodes the copper-transporting ATPase 2; this enzyme is required for the release of copper from cells and the loading of blood ceruloplasmin with copper, thereby enhancing the excretion of copper via the bile. ATPase 2 is mainly expressed in the liver and to a lesser extent in the brain and kidneys. Dysfunctional ATPase 2 causes marked sequestration of copper in cells of a number of tissues, including the liver and brain [3]. In these and other tissues, toxic levels of copper, due to malfunction of copper-transporting ATPase, mediate cellular damage. In hepatocytes, the presence of excess copper leads to the generation of oxidants, e.g., reactive oxygen species (ROS), which destroy normal molecules and cause high levels of oxidative stress, contributing to the progression of WD [4].

Within eukaryotic cells, copper is mostly stored in the mitochondrial matrix [5], ranging from 30 to 50 ng/mg mitochondrial protein [6], given the need for cytochrome c oxidase function to meet energy requirements. Excess copper in liver cells leads to structural, biophysical and biochemical alterations in mitochondria, [6] with an increase in abnormal mitochondrial structures and altered protein thios that correspond to increased copper burden [6]. Mitochondrial membranes become less stable and lose their flexibility that consequently also leads to defective electron transport chain [7]. Overall, ATP production decreases and ROS-mediated oxidative damage increases with increasing levels of copper [8]. Importantly, treatment with penicillamine restores mitochondrial function and consequently, rescues liver damage [8]. Therefore, mitochondria appear to be key players involved in cellular toxicity to copper.

In addition to the effects on mitochondria, morphological and functional damage to the endoplasmic reticulum (ER), referred to as ER stress, is also observed, as indicated by the large increases in misfolded...
and abnormal proteins. Oe and colleagues [9] reported markedly exaggerated ER stress in a human hepatoma cell line (HuH7) and an immortalized hepatocyte cell line (OUMS29) treated with copper. Copper exposure also promoted DNA damage, diminished hepatocyte proliferation and enhanced apoptosis.

Treatments for Wilson disease

Conventional medication used to treat Wilson disease are chelating agents that bind tissue copper; this leads to the release of sequestrated copper from cells into the bloodstream, allowing it to eventually be excreted via the bile. The treatment is then directed towards reducing the re-accumulation of tissue levels of copper. Once the use of medications for WD is initiated, it is continued over the course of the life span of an individual.

The most common medication is the copper chelating agent, penicillamine (prescription drugs, Cuprimine® or Depen®). These drugs have complex and subtle side effects leading to pathophysiological changes in the kidney and skin [10]. Also, they sometimes aggravate the neurological symptoms associated with WD and bone marrow suppression has occurred. Trientine hydrochloride (Syprine®) has similar functions to penicillamine but usually with fewer negative consequences; however, the use of this drug may worsen the neurological deficits of WD [11].

After the systemic redirection of copper levels with the drugs mentioned, zinc acetate (Galzin®) may be a useful maintenance therapy to reduce copper absorption from the diet [11]. Once extensive damage to the liver occurs, the liver may become cirrhotic which contributes to hepatic failure [12]. With a severely damaged liver, the only option remaining is a liver transplant although this is rare in individuals suffering with WD.

The use of melatonin as a test molecule to treat Wilson disease

Melatonin (N-acetyl-5-methoxytryptamine) is an endogenously-produced molecule which is present in every vertebrate species. Its best known site of synthesis is the pineal gland where it is most abundantly generated during the daily dark period after which it is quickly released into the blood and cerebrospinal fluid causing a large nighttime rise in these fluids [13]. Additionally, however, this critically important agent was recently proposed to be produced in the mitochondria of every cell [14], consistent with its close functional association with these organelles [15,16].

Recent reports strongly support the synthesis of melatonin in mitochondria of all cells [17,18]. Melatonin is also considered a mitochondria-targeted agent when parenterally administered [19,20]. Besides its production in mitochondria, the incubation of cells containing melatonin [21,22] or its exogenous administration to animals causes its rapid accumulation in all subcellular compartments, especially in mitochondria [23]. Herein, we review the functions of melatonin in copper chelation, antioxidant activity and ER stress inhibition that justify tests in models of WD.

Melatonin as a copper chelator

Penicillamine, the most commonly used drug to treat WD, is a copper chelator which allows the release of copper from cells and its excretion into the bile [10]. Limson and colleagues [24] were the first to test the binding affinity of copper with melatonin. With the aid of absorptive stripping voltammetry, they reported that melatonin did bind Cu²⁺ and several other metals; similarly, melatonin’s precursor, serotonin, also formed a complex with copper. In a subsequent in vitro study, melatonin reduced copper-mediated lipid peroxidation in hepatocytes [25]. This was also noted in an in vivo study where Cu²⁺ (as cupric chloride) distorted the ultrastructure of hepatic cells, changes ameliorated by concurrent administration of melatonin. While the protection provided by melatonin against copper-mediated lipid peroxidation may have been due to its binding of Cu²⁺.

Fig. 1. Melatonin and several of its metabolites function as highly effective scavengers in all cells. This figure illustrates the antioxidant cascade mediated by direct scavenging of free radicals by melatonin and its metabolites. The ability of each of these molecules to detoxify radicals and reduce oxidative stress has been verified after the initial description of this series of reactions.

Thus, melatonin may shield cellular molecules including lipids from oxidation due to its ability to function as an antioxidant (see below).

In the most comprehensive study performed to date, Galano and colleagues [26] compared melatonin as well as its metabolites, which are formed during the radical scavenging cascade of melatonin, in terms of their ability to neutralize Cu²⁺. The melatonin metabolites studied included cyclic 3-hydroxymelatonin (c3-OHM), N¹-acetyl-N²-formyl-5-methoxykynuramine (AFMK) and N¹-acetyl-5-methoxykynuramine (AMK) (Fig. 1); the comparisons were done in the framework of Density Functional Theory. Each of the molecules examined yielded stable complexes with Cu²⁺. Also, each of the compounds prevented free radical damage that occurred in a Cu²⁺-ascorbate mixture and reduced hydroxyl radical generation by interrupting the initial phase of the Haber-Weiss reaction. The results confirm that melatonin is an effective Cu²⁺ chelator and show, for the first time, so are several of its major metabolites. While copper chelation is an important function of melatonin and drugs conventionally used (e.g., penicillamine) to treat WD, whether melatonin also aids the biliary excretion of copper, as does penicillamine, has yet to be determined [27].

Melatonin as an antioxidant

As mentioned in the previous section, melatonin limits molecular damage due to copper toxicity by neutralizing free radicals, i.e., it functions as an antioxidant [28,29]. There is a vast amount of literature documenting the ability of melatonin, as well as several of its metabolites, to potently reduce oxidative stress [30,31]. This may involve the direct detoxification of reactive oxygen species (ROS) and/or the stimulation of antioxidant enzymes [32,33]. Melatonin has been shown to strongly protect the liver and brain, the two organs most markedly damaged in WD, from oxidative damage resulting from a large variety of free generating processes/agents, e.g., metals [34], alcohol [35], ionizing radiation [36] and drugs [37].

Specifically, with regard to copper toxicity, melatonin reduced molecular damage to cardiac mitochondria [38] and alleviated copper-mediated oxidative stress in plants [39,40]. Oxidative damage to hepatic and neural tissues is a feature of WD. Moreover, chronic treatment
with penicillamine, the drug developed to reduce oxidative stress and neurological damage in WD, causes new lesions in the white matter, thalamus, midbrain, andpons (evaluated by magnetic resonance imaging); these neurodegenerative changes are accompanied by elevated serum copper and malondialdehyde, a product of lipid peroxidation, and reduced glutathione concentrations. These changes resolve after penicillamine treatment is withdrawn [4,10]. This indicates that the drug designed to protect against neural damage in WD may, in some cases, worsen the condition. In animal studies, melatonin has not been shown to exaggerate drug-mediated oxidative damage to tissues. Although the combination of melatonin with penicillamine has not yet been studied, we estimate that a reduction in neurotoxic effects with more significant benefits to patients would be highly likely due to synergism [41].

One well-known antioxidant (Co-enzyme Q10) has been tested for its potential efficacy as a treatment for neurological diseases but it failed to have benefits [42]. Q10, being a large molecule, is not as efficiently targeted to the mitochondria as is melatonin, the highest concentrations of which are found are in the mitochondria [43]. Targeting of an antioxidant to the mitochondria is critical to its efficacy as an antioxidant since these organelles are a primary site of free radical generation [44,45]. However, coupling of Q10 to the triphenylphosphonium ion cation increases its accumulation in the mitochondria (MitoQ). More than a decade ago, vitamin E was proposed as a treatment for Wilson’s disease [46]. No one has followed up on that suggestion; while it is not targeted to the mitochondria, coupling of α-tocopherol to the triphenylphosphonium cation increases its accumulation in the mitochondria (MitoE). Both, MitoQ and MitoE, appear to be as effective as melatonin in equimolar concentrations in reducing oxidative stress in experimental animals [44]. However, unlike MitoQ or MitoE, melatonin also promotes the expression of other antioxidants within the cell [47,48]. Moreover, these compounds do not manifest Cu²⁺ chelation, an important feature of a treatment for WD.

Recently, it has been noted that gut flora in WD patients show an abundance of Prevotella species which are pro-inflammatory [49]. Gut bacteria are known to release short chain fatty acids (SCFA) such as butyrate, propionate and acetate that play an important role in reducing inflammation and enhancing mitochondrial function during oxidative stress [50]. Importantly, butyrate has been shown to induce arakylamine N-acetyltransferase (AANAT), acetylsertotonin O-methyltransferase (HOMT) and N-acetylserotonin that results in melatonin synthesis in vivo in mice [51]. Indeed, most of butyrate’s effects on mitochondrial function, inflammation and autophagy parallel those of melatonin [52]. Taken together, it is possible that melatonin plays a central role in gut microbiome-mediated effects of butyrate on the mitochondria [52].

**Melatonin as an ER stress inhibitor**

The endoplasmic reticulum (ER) is the major subcellular site for proper folding and maturation of newly-synthesized proteins. These processes take place in the lumen of the ER. A variety of factors can cause proteins to fail to mature or to become misfolded; these conditions include genetic errors, environmental influences, and exposure to toxins. When unfolded or misfolded proteins accumulate in the ER beyond a critical level, ER stress occurs which initiates an ER to nucleus signaling pathway termed the unfolded protein response (UPR). The UPR protects cells from stress by maintaining cellular homeostasis. However, when UPR persists, it can lead to cell death via apoptosis. ER stress and cell death are observed in both the brain and liver of individuals with WD [3,4].

Melatonin is documented to suppress neural ER stress and the UPR under conditions of drug or toxin exposure [53,54]. Given that the mechanisms of ER stress are common to many situations, it is reasonable to suggest that melatonin would likely reduce ER stress in the brain of subjects with WD. Melatonin inhibits free radical-mediated ROS and inflammation in the CNS, both of which are preludes to ER stress [55]. Inflammation is associated with the generation of ROS.

In the liver as well, melatonin plays a key role in reducing ER stress induced by fulminant hepatitis of viral origin, by rabbit hemorrhagic disease virus as well as other causes [56-58]. Moreover, melatonin suppressed ER stress-mediated hepatic steatosis via its action on the microRNA, miR 23a [59]. Steatosis is a common feature of WD [60,61]. Again, given the capacity of melatonin to forestall ER stress [62] and subsequent downstream events such as autophagy and apoptosis, resulting from such a diversity of promotors, it is possibly that melatonin supplementation to WD patients would protect against hepatic degeneration and liver failure.

**Melatonin as an anti-fibrotic agent**

Severe hepatocyte injury is commonly accompanied by the development of fibrosis/cirrhosis, an overabundance of fibrous connective tissue [3,63,64]. Fibrosis results from the hyperactivity of fibroblasts/myofibroblasts which produce collagen I. In the short term, fibrosis is presumed thought to be a reparative response, but when exaggerated the massive amount of connective tissue becomes pathological [64]. With persistent fibrosis/cirrhosis, cellular function is compromised and organ failure results [65]. This frequently occurs in many conditions that destroy hepatocyte physiology, e.g., alcoholic liver disease, non-alcoholic liver disease, toxin and drug metabolism, etc. Excessive collagen I accumulation is also characteristic in the advanced stages of WD [60,61]. While mild fibrosis is reversible, severe fibrosis is irreversible; a liver transplant is the only reliable solution although this is rare in WD. Fibrotic pathogenesis is a consequence of a number of overlapping processes that include infection, the associated inflammatory response and free radical generation with resulting oxidative stress [65,66].

Clearly, preventing collagen I build-up is essential to avoid fibrosis in any organ. Melatonin is capable of limiting excessive collagen I formation in multiple organs due to many different circumstances [63]. In the liver, the primary cells that are critical to initiating and sustaining the fibrotic cascade are the hepatic stellate cells [67]. The development of fibrosis is always preceded by molecular damage caused by free radicals that were not neutralized. Since melatonin and its metabolites are highly effective in detoxifying these oxidizing agents, they reduce liver cirrhosis via their ability to limit oxidative damage [65,66]. Many studies have confirmed that melatonin inhibits the majority of agents responsible for the fibrotic cascade, i.e., transforming growth factor-β (TGF-β), connective tissue growth factor (CTGF) and platelet derived growth factor (PDGF) [64]. These along with SMA and MAD Related Protein 3 (SMAD3), are all markers of the profibrotic pathway [67]. Given these results and those documenting that the final product, i.e., collagen, which contributes to hepatic fibrosis and cirrhosis is strongly inhibited by melatonin, it is likely that melatonin would also reduce collagen I production in the liver of WB patients.

**Melatonin administration to humans**

Melatonin has few side effects at any dose and it has not been possible to establish an LD50 in animals (lethal dose for 50% of the animals), although attempts have been made to do so [68]. This indoleamine has been widely tested as a sleep promoting agent [69,70]. The doses used in these studies (usually 5 mg daily or less) would seemingly be insufficient to alter the course of WD. Much larger doses of melatonin have been given to humans with no reports of noticeable toxicity [68]. One gram of melatonin was given to adult humans daily for one month with the expressed purpose of identifying toxic reactions; no measurable abnormalities were found [71]. Other human studies have used daily doses of 300 mg/day for two [72] or four years [73] without significant side effects. Gatto et al. [74] treated premature human newborns suffering with severe sepsis with 80 mg intravenously
and reduced lipid peroxidation and prevented death of these seriously-ill patients; no short-term or long-term side effects were noted in this study [74].

The specific amount of melatonin that would be required to stall the progression of WD is unknown. In an Alzheimer patient, Brusco et al. [75,76] found that 6 mg daily for 3 years deferred to progress of the disease by improving sleep quality, reducing signs of sundowning and limiting brain shrinkage. In the studies where melatonin was tested against neurological diseases, the patients always showed improvement [68]. An important point to consider would be the gender of the patient. Men predominantly suffer from WD and exhibit the neuropsychiatry form of WD, characterized by cerebellar atrophy and reduced lipid peroxidation and prevented death of these seriously-ill patients; no short-term or long-term side effects were noted in this study [74].

The specific amount of melatonin that would be required to stall the progression of WD is unknown. In an Alzheimer patient, Brusco et al. [75,76] found that 6 mg daily for 3 years deferred to progress of the disease by improving sleep quality, reducing signs of sundowning and limiting brain shrinkage. In the studies where melatonin was tested against neurological diseases, the patients always showed improvement [68]. An important point to consider would be the gender of the patient. Men predominantly suffer from WD and exhibit the neuropsychiatry form of WD, characterized by cerebellar atrophy and reduced lipid peroxidation and prevented death of these seriously-ill patients; no short-term or long-term side effects were noted in this study [74].

The specific amount of melatonin that would be required to stall the progression of WD is unknown. In an Alzheimer patient, Brusco et al. [75,76] found that 6 mg daily for 3 years deferred to progress of the disease by improving sleep quality, reducing signs of sundowning and limiting brain shrinkage. In the studies where melatonin was tested against neurological diseases, the patients always showed improvement [68]. An important point to consider would be the gender of the patient. Men predominantly suffer from WD and exhibit the neuropsychiatry form of WD, characterized by cerebellar atrophy and reduced lipid peroxidation and prevented death of these seriously-ill patients; no short-term or long-term side effects were noted in this study [74].

Conclusions and perspective

Considering the identified causes of molecular damage to hepatocytes and cells in the CNS which contribute to WD, we anticipate melatonin, via its multiple actions as described herein, may be beneficial in delaying the progression or alleviating the symptoms of this condition (Fig. 2). There are animal models of WD where this could be tested. The actions of melatonin (and of its metabolites, which are formed when melatonin functions as a direct antioxidant) include the following: high capacity to bind copper, potent antioxidant activity (direct radical scavenging, stimulating antioxidant enzymes, inhibiting pro-oxidant enzymes), anti-inflammatory actions, ability to suppress ER stress and anti-fibrotic activities.

In many experimental and clinical situations, these functions of melatonin have been documented. Moreover, with reference to the brain, melatonin readily crosses the blood/brain barrier and prevents damage to the barrier induced by other drugs/toxins. Finally, when administered, melatonin, especially concentrates in mitochondria [23], a major site of oxidative stress contrast, the current drugs used to treat WD are not totally effective and have side effects. It seems likely that melatonin may alter the trajectory of WD and improve the quality of life for these individuals, as it has in other clinical situations.

The binding of copper by melatonin, along with its other actions, may in part, also account for its reported beneficial effects of melatonin in models of other neurodegenerative diseases, e.g., Alzheimer disease [80–82], Parkinson disease [80,83,84], amyotrophic lateral sclerosis [85–87], and multiple sclerosis [88,89]. Melatonin could be used as a sole treatment or in combination with other drugs, e.g., penicillamine, butyrate. Judging from the published literature, melatonin would, perhaps, reduce the toxicity and increase the efficacy of associated medications [37].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.109408.

References


