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Melatonin in relation to the "strong" and "weak" versions of the free radical theory of aging

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ABSTRACT

That free radicals and the damage they inflict are related to deteriorative cellular and organismal changes associated with aging and also with the development of a variety of age-related diseases is widely debated. There seems to be little doubt that free radical mutilation of essential molecules contributes to these conditions. Numerous investigators, on the basis of their experimental results, have drawn this conclusion. If the free radical theory of aging and disease development has validity, antioxidants could presumably be successfully used to delay the molecular destruction, cellular loss, and organismal death. In the current review we summarize the experimental data related to the utility of melatonin in protecting against reactive oxygen and reactive nitrogen species-induced cellular damage. While the data supporting a role for melatonin in forestalling aging and prolonging life span per se is not compelling, the findings related to melatonin's ability to reduce the severity of a variety of age-related diseases that have as their basis free radical damage is convincing. To date, the bulk of these investigations have been performed in experimental models of diseases in animals. It is now imperative that similar studies be conducted using humans whose quality of life may benefit from treatment with melatonin.

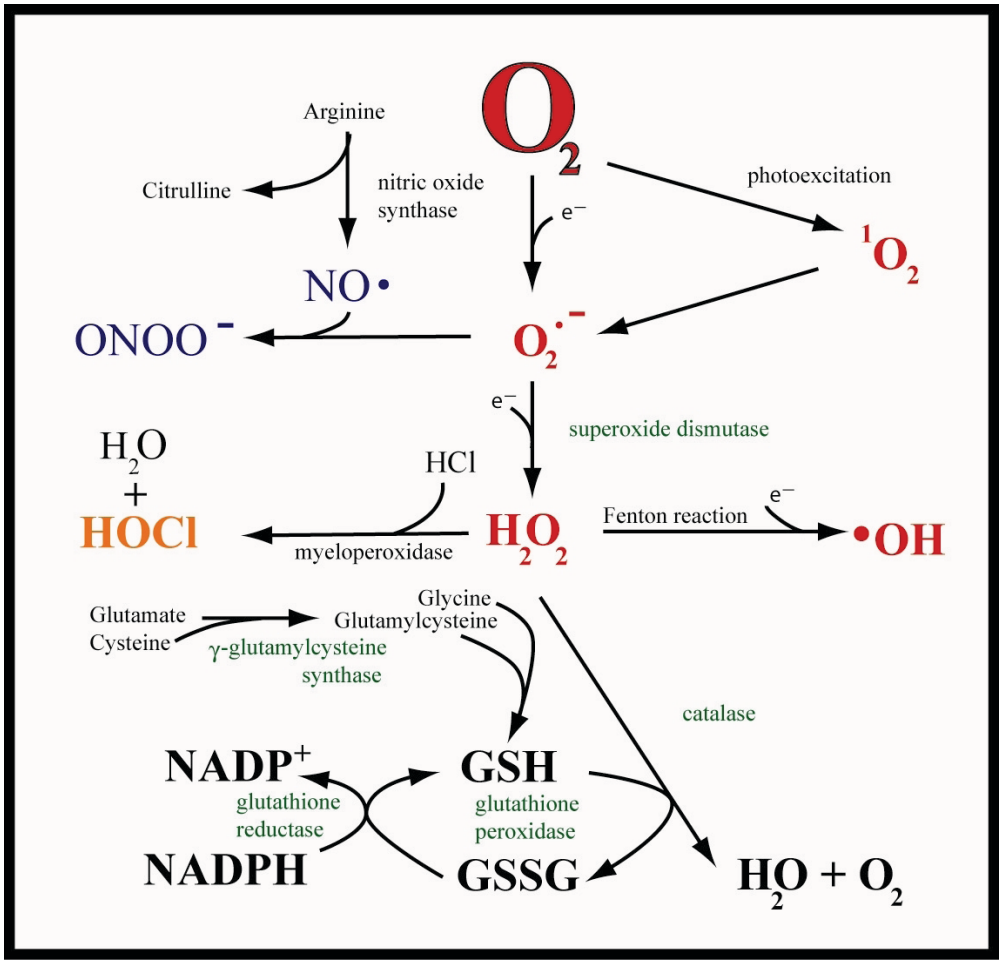
Key words: melatonin, aging, life span, neurodegenerative diseases

INTRODUCTION

The free radical theory of aging (also known as the oxidative stress theory) has a long history, having been formulated by Denham Harman in 1956 [1]. Based on this theory, organismal deterioration that occurs as a result of increasing longevity is specifically a consequence of the persistent accumulation of free radical-mediated damage to essential molecules that gradually compromise the function of cells, of tissues and eventually of the organism itself. Since it was proposed, the free radical theory of aging has been repeatedly modified and re-fined [2,3]. Special attention has been focused on the mitochondria since they are a major site of oxygen-based free radicals and related non-radical species [4]. Conventionally, the radical and non-radical molecules that are oxygen-based are referred to as reactive oxygen species (ROS). Besides ROS, however, some toxic reactants are nitrogen-based and are collectively referred to as reactive nitrogen species (RNS). Both ROS and RNS indiscriminately mutilate molecules in the area of where they are generated.

Within the last decade the free radical theory has been subdivided into what is referred to as a "strong" and "weak" versions of the hypothesis [5]. The "strong" version states that accumulated oxidatively-induced molecular debris determines the lifespan of an organism; thus, when the oxidative burden becomes excessive, the animal dies. The "weak" version of the oxidative stress theory postulates that oxidative damage is causative of or associated with age-related diseases. Indeed, there are numerous diseases/conditions that develop in aged individuals that have, at least as part of their basis, free radical damage [5-8]. These conditions eventually compromise the health of an organism and, ultimately, death follows. Thus, according to the "weak" version of the free radical theory of aging, ROS/RNS only secondarily determine lifespan. Obviously, there is a continuum between the "strong" and "weak" versions of the hypothesis. In general, there is more experimental evidence supporting the "weak" version than there are data consistent with the "strong" version. Alternative terms to describe the "strong" and "weak" versions of the

Figure 1. **Oxygen (O_2) is a major source of free radicals and related oxidants. Once the superoxide anion ($O_2^{\cdot-}$) is formed it can either generate additional oxygen-based reactants, e.g., H_2O_2 and the •OH, or it can form the nitrogen-based reactant, $ONOO^-$, after it couples with NO•. Also shown are the antioxidative enzymes which melatonin stimulates (glutathione peroxidase, glutathione reductase, catalase, superoxide dismutase and γ-glutamylcysteine synthase) and the prooxidative enzyme (nitric oxide synthase) which it inhibits.**



theory may be "direct" and "indirect". The former directly impacts longevity while the latter indirectly influences life span.

The current review will summarize some of the data which support both the "strong" and "weak" versions of the oxidative stress theory of aging. Moreover, this survey will consider the role of melatonin in these conditions.

Oxidative/Nitrosative Stress as a Life-determining Factor

Oxidative stress is a result of damage to essential molecules by ROS that are obviously oxygen-based whereas nitrosative stress is molecular destruction that occurs as a consequence of nitrogen-based reactants or species, i.e., RNS. However, in reality when mutilated molecules are detected in vivo it is often difficult to determine whether they are a consequence of ROS or RNS, i.e., whether it is oxidative or nitrosative stress. The reason for this difficulty stems from the fact that the generation of oxygen and nitrogen-based reactants are intertwined.

Once molecular oxygen is reduced by one electron to the superoxide anion radical ($O_2^{\cdot-}$) it can be subsequently involved with additional ROS generation when it is dismutated. Additionally, however, the $O_2^{\cdot-}$ can couple with nitric oxide ($NO^{\cdot}$) to form the highly toxic nitrogen-based reactant, the peroxynitrite anion ($ONOO^-$). The latter molecule is believed to degrade into the hydroxyl radical ($^{\cdot}OH$), a highly reactive oxygen-based agent. These interrelationships of the oxygen and nitrogen-based damaging agents are summarized in *Fig. 1*.

That ROS/RNS damage occurs in vivo has been routinely documented in virtually all species [4]. One means of inflicting such damage is to expose organisms to an elevated oxygen tension, referred to as oxygen "poisoning" [9]. Use of this procedure in early experiments, in fact, assisted Harman [1] in formulating the free radical theory of aging. Molecular oxygen (O_2), while obviously being necessary for survival of all aerobic organisms, can also be deadly; this is primarily due to the fact that during its use at the mitochondrial level, i.e., the successive metabolic reduction of O_2 in the electron transport chain, numerous free radicals are generated leading

to a reduced function and/or complete destruction of the mitochondria. This mutilation, in many cases, can induce death of the cell via programmed cell death or apoptosis [10,11]. As cells die in increasing numbers, the functional aspects of an organ deteriorate and death of the organism can follow.

The ability of oxygen "poisoning" to shorten life span has been documented in the fruit fly (*Drosophila*) [12]. Thus, elevating O_2 tension above the normal value, i.e., 21%, correspondingly reduced the survival time of this species. For example, at an oxygen tension of 40% life span was reduced by 30%. In contrast, however, reducing oxygen tension below 21% is without influence, i.e., it does not increase the lifespan of the fruit fly. Perhaps this is not surprising given that hypoxia, which is obviously a consequence of depressed oxygen tension, also leads to an increased ROS production.

If, in fact, the reduced survival of *Drosophila* exposed to an elevated O_2 atmosphere is due to oxygen "poisoning" or a consequence of authentic accelerated aging is difficult to determine. If the latter is the case, then the observations would support the "strong" version of the oxidative stress theory of aging.

Genetic manipulations of the fruit fly also provide evidence that ROS/RNS are involved in determining life span. Based on what was currently known, it was anticipated that the over expression of the genes for the cytoplasmic and mitochondrial isoforms of superoxide dismutase (*Sod1* and *Sod2*), respectively, would reduce oxidative damage (although there is not universal agreement on this point) and promote increased longevity. When the *Sod1* gene was over expressed in fruit flies, the results relating to the duration of survival were inconsistent; in some cases survival was prolonged [13,14]. That life span was not uniformly increased in these studies was perhaps not unanticipated since when SOD converts the $O_2^{\cdot-}$ to hydrogen peroxide (H_2O_2); the latter molecule, if it is not enzymatically metabolized to innocuous products, is available for conversion to the devastatingly toxic ·OH. This is certainly further supported by the findings of Sohal and co-workers [15,16] who showed that the concomitant over expression of both *SOD* and the gene for the catalase enzyme (which enzymatically converts H_2O_2 to water and O_2) did indeed lead to life extension of fruit flies. Even this observation, however, does not provide definitive proof of the "strong" version of the oxidative stress theory of aging since in the transgenic studies the control and experimental flies had a different genetic makeup which could have independently influenced their life span.

Collectively, if all the data related to the genetic manipulation of genes that metabolize ROS to harmless byproducts are examined and evaluated, the findings indicate that $O_2^{\cdot-}$ generation and metabolism may well determine longevity in the fruit fly. Elevation of these essential antioxidative enzymes generally promotes increased survival.

Another invertebrate that has been rather extensively used to test the free radical theory of aging is *Caenorhabditis elegans* [17-19]. These studies have centered on the *dauer* mutant of this species; because of point mutations in the mitochondrial electron transport chain, which lead to elevated $O_2^{\cdot-}$ production, the mutants have a reduced life span [20]. However, a partial inhibition of Complex III in the mitochondrial respiratory chain, also extended life span; this argues against the free radical theory of aging given that the Complex III inhibitor used, i.e., antimycin, actually significantly elevates the longevity of this worm. This argues against the theory since antimycin increases $O_2^{\cdot-}$ production and presumably the levels of other toxic species as well.

Mouse models which have enhanced or depressed levels of antioxidative enzymes have also been used to test the relationship of oxidative stress to longevity. Elevated activities of antioxidative enzymes would generally be considered protective against free radical damage and, therefore, they would preserve survival; conversely, depressed activities of these enzymes would be expected to have the opposite effect [21,22].

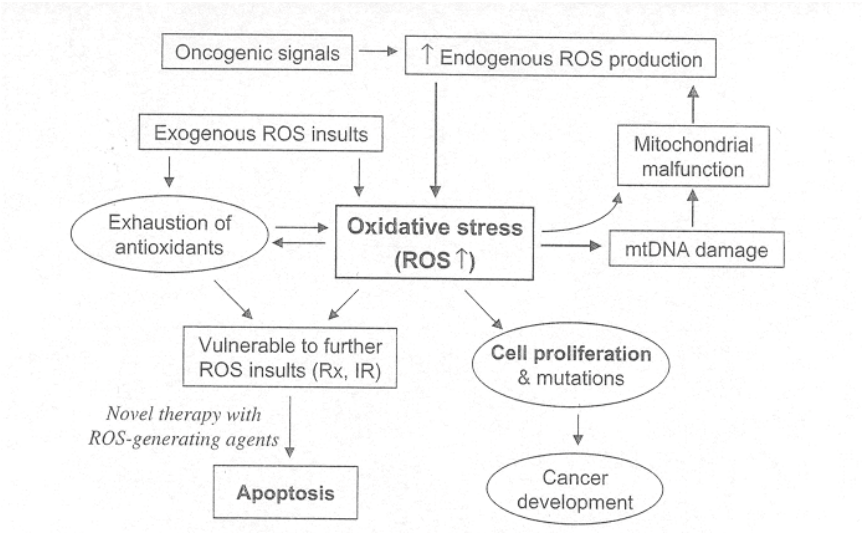
Since the mitochondria are major sites of production of the $O_2^{\cdot-}$, the genetic ablation of mitochondrial SOD (MnSOD) might significantly influence lifespan and, indeed, it does. In mice where this enzyme is genetically removed, these animals die within 24 days after birth and furthermore, virtually all parameters of oxidative damage are elevated [23]. Additionally, these animals exhibit an increased sensitivity to elevated oxygen tension. Again, while these findings do not prove beyond a doubt that free radical damage accounted for the abbreviated life span of the mice, the data are certainly consistent with this hypothesis.

In contrast to MnSOD which is located in mitochondria, CuZnSOD, also a $O_2^{\cdot-}$ scavenger, is situated in the cytoplasm and in the mitochondrial intramembrane space. While mice in which the gene for CuZnSOD has been genetically deleted do not exhibit any remarkable deleterious phenotypes that lead to an abbreviated life span, there are molecular and functional deficiencies that could relate to augmented free radical damage.

The primary scavenging enzymes of H_2O_2 are catalase and the glutathione peroxidases, which is located both in the mitochondrial and cytoplasmic compartments of the cell. Genetic ablation of only catalase is essentially inconsequential in terms of accumulated free radical damage or life span in mice [24]. Likewise, knockout mice deficient in glutathione peroxidase only do not exhibit marked changes in either the degree of oxidative stress or life span [25] although they do show premature cataract formation, a condition generally considered to be caused by free radicals. Generally, these findings may not be unexpected since one could anticipate that the loss of catalase activity would be compensated for by glutathione peroxidase and, vice versa, since both enzymes remove H_2O_2 from cells.

Presumably because antioxidative enzymes often have overlapping and/or complementary functions, knockouts of genes of single enzymes have not very rewarding in documenting a role for free radicals in determining life span

Figure 2. **Relationship of oxidative stress to cancer initiation and progression. Nuclear DNA, once damaged by free radicals, can mutate which leads to the initial growth of cancer cells. Once initiated cancer cell proliferation can be enhanced by free radicals.**



in either invertebrates or vertebrates. Combination knockouts involving two or more antioxidative enzymes are ongoing but interpretation of the results will likely be difficult because of the differences in the genetic background of the control and experimental animals.

Overall, the evidence supporting what is referred to as the "strong" version of the free radical theory of aging is not indisputable. Thus, whether the free radical damage accumulated throughout a life time directly determines how long an individual animal survives has yet to be proven. Despite this uncertainty, the theory as originally proposed by Harman [1] is important for the mere fact that it has instigated a large amount of research that has contributed to our knowledge related to oxidative stress and disease initiation and progression.

Oxidative/Nitrosative Stress as a Disease-determining Factor

The "weak" version, i.e., that persistent mutilation of cellular molecules and organelles leads to age-associated diseases that may coincidentally reduce life span, of the free radical theory of aging has significantly more support than what is referred to as the "strong" version. Even in humans there is evidence supporting this possibility since the portion of the population that eats the fewest fruits and vegetables, which are rich in antioxidants, reportedly have approximately twice the cancer rate as individuals who commonly consume such foods [26]. Although there are exceptions, most often cancer is a late-life development and free radical damage-dependent.

It is estimated that 60-70% of the cancers that are initiated are initially a consequence of the nuclear DNA taking a hit from a free radical [27]. Whereas the resulting damage that occurs is sometimes repaired, if not, a mutation can occur and a cancerous growth may be initiated (*Fig. 2*). Interestingly, cancer cells themselves produce higher levels of ROS/RNS than do normal cells indicating that these cells are being continually bombarded by free radicals and associated reactants. To make matters worse, cancer cells are often deficient in antioxidative enzymes, e.g., MnSOD [28].

ROS produce multiple forms of damage in DNA including base modifications, loss of a base (apurinic/apyrimidinic site), single and double strand breaks, DNA-protein cross-links, and the oxidation of deoxyribose. The majority of this damage is specifically a result of the ·OH. Among many subcellular changes that occur as a result of free radical-mediated DNA damage is interference with cell-to-cell communication via gap junctions. It is postulated that blockade of intercellular communication may provide preneoplastic cells with a selective advantage since they would no longer be under the growth regulatory effect of the surrounding normal cells [29].

In disease states where ROS/RNS generation is elevated, cancer is also a common occurrence. Some conditions in which the frequency of cancer is common include Fanconi anemia, ataxia telangiectasia, xeroderma pigmentosa, Bloom syndrome, Down syndrome, etc. While it is often hypothesized that the tumors in these individuals are a result of free radical-mediated DNA damage, this has not been easy to prove.

The central nervous system (CNS) is particularly susceptible to destruction by toxic derivatives of O_2. There are a number of reasons for the increased vulnerability of the brain. First and foremost, the CNS, although averaging only 2% of the body weight, consumes an estimated 20% of the inhaled O_2; as a result, radicals and radical products derived from O_2 in the brain are markedly elevated [30]. Compounding the elevated free radical production is a deficiency of antioxidative enzymes in neural tissue. Additionally, the brain contains high levels of potential pro-oxidants, ascorbate (vitamin C) and iron. If liberated from cells due to damage, these molecules cooperate in the generation of the highly reactive ·OH. Finally, most neurons are postmitotic so once damaged or destroyed,

they are not restored. As important as the CNS is to optimal functioning of the organism, it might be predicted that it would be well protected against ROS/RNS. Unfortunately, the reverse situation seems to be the case [31].

The loss of memory, alertness, balance, discrete movements, coordination, etc., are all consequences of aging which likely result from the reduction in the number of neurons that are commonly associated with increased longevity. Additionally, there are some specific, age-related diseases of the brain that have, at least in part, a free radical component. Some of the most obvious and debilitating neurodegenerative conditions that may involve the destruction of brain cells by ROS/RNS include Alzheimer disease (AD), Parkinson disease (PD), ischemia/reperfusion (IR) injury (stroke), amyotrophic lateral sclerosis (ALS), etc. Each of these conditions generally manifests an increased frequency with advancing age [30].

In reference to the pathogenesis of AD, both the rare familial form of the disease as well as the more common sporadic form are considered to involve oxidative stress linked to amyloid-β toxicity and neurofibrillary tangle formation. PD is characterized by the progressive and selective loss of dopaminergic neurons in the pars compacta of the substantia nigra [32]. The reason for the selective vulnerability of these neurons to destruction is usually assumed to be due to oxidative damage since the biosynthesis and metabolism of dopamine produces $O_2^{\cdot -}$ and H_2O_2. The damage inflicted initially seems to be at the mitochondrial level which leads not only to the generation of an excessive load of ROS/RNS but also to a reduction in energy production in the form of ATP. In reference to ALS, the progressive degeneration of upper and lower motorneurons in the cerebral cortex and spinal cord, respectively, is associated with evidence of the involvement of exaggerated oxidative stress given the elevated levels of oxidatively-modified proteins, DNA and lipids [33]. Finally, IR injury of the CNS, like IR damage in other organs, includes tissue destruction induced during both the hypoxic/ischemic period as well as at the onset of reperfusion.

A variety of aspects of cardiovascular pathophysiology are influenced by ROS/RNS [34]. Endothelial cells are a major regulator of vascular homeostasis with NO being a primary factor in the regulation of vasodilatory tone. Inactivation of $NO\cdot$ can occur by its rapid diffusion-limited reaction with $O_2^{\cdot -}$ to produce the high reactive $ONOO^{\cdot}$ [30]. This latter agent causes cellular damage that contributes to cardiovascular dysfunction and, along with other toxic reactants, it influences blood pressure, the progression of atherosclerosis, heart failure, cardiac hypertrophy and myocardial infarction. The cardiovascular changes described increase in frequency as individuals age.

It is obvious from this brief discussion that a variety of diseases in the elderly involve directly or indirectly excessive free radical damage. Thus, what is referred to as the "weak" free radical theory of aging has abundant support. Since these diseases are frequently life threatening, they often also reduce life span.

Melatonin in Relation to the "Strong" Version of the Free Radical Theory of Aging

It is thoroughly documented that melatonin and its metabolites have both direct scavenging actions against free radicals and related products [35-38] as well as indirect antioxidative actions [39,40] via its ability to stimulate antioxidant enzymes, to inhibit the prooxidative enzyme nitric oxide synthase [41], to promote the synthesis of another important intracellular antioxidant, glutathione [42,43], and to diminish, free radical formation at the mitochondrial level by reducing the leakage of electrons from the electron transport chain [44]. Additionally, melatonin synergizes with other antioxidants to protect against oxidative stress. This combination of actions makes melatonin an important agent in combatting some signs of aging and/or the initiation of age-related diseases.

Experimental evidence that melatonin per se defers aging and, by extension, relates to the "strong" version of the free radical theory of aging is not convincing at this point. Rather few investigators have undertaken, in a meaningful way, experiments related to melatonin administration or deprivation and longevity. What has enticed researchers is that endogenous melatonin production wanes with increasing age leading some to speculate that its loss contributes to the aging process [45,46]. This supposition was also based on the numerous beneficial effects that supplemental melatonin displays in terms of seemingly forestalling some signs of age-related deterioration.

In the unicellular protozoan, *Paramecium tetramelia*, the addition of melatonin to the medium in which they were growing increased both the mean and maximal clonal life span by 25% [47]. Likewise, in aquatic rotifers a similar prolongation of survival was documented in both short-lived and long-lived species [46]. Finally, in the fruit fly, a 10-20% or greater increase in life span was reported after feeding them the indoleamine in their diet [48]. In contrast, however, melatonin did not exaggerate the life span of *C. elegans*.

In mammals, the accumulated results related to melatonin and life extension are contradictory [46]. While there are reports of prolonged life span in some rodents after melatonin administration [49,50], there are an equal number of studies claiming no effect. Moreover, in general it seems that male mice benefit more than females in terms of longevity when melatonin treatment is prolonged [51] although it is premature to make this generalization. In rats, Oaknin-Bendhan et al [52] claimed that providing melatonin in the drinking water (only males were studied) resulted in up to 50% prolongation of life span. The outcome of this study was confusing, however, since giving a melatonin receptor antagonist, ML-23 (N-(2,4-dinitrophenyl-5-methoxytrptamine), also was associated with an increased life span.

The senescence accelerated mouse (SAM) has been used to test whether melatonin modifies the aging process in this relatively short-lived strain. While melatonin clearly has benefits in terms of enhancing mitochondrial physiology [53], slowing the rate of accumulation of molecular garbage

resulting from free radicals [54], and improving the immune responsiveness of SAM [55], that the indoleamine actually significantly improves life span has not been unequivocally documented. Likewise, in rats melatonin also has beneficial antioxidative effects in old animals but whether longevity is changed as a result of the treatment is yet undetermined [56,57].

In general, in regard to the role of melatonin in the "strong" version of the free radical theory of aging, the (i) data are not especially consistent, (ii), the number of animals used was typically small, and (iii), the number of studies is too few on which to base any substantial conclusion.

Melatonin in Relation to the "Weak" Version of the Free Radical Theory of Aging

The published literature purporting to show an association between melatonin and debilitating diseases frequently associated with advancing age are not only in general agreement but also quite numerous [58-64].

Considering the outcomes of a large variety of published papers, the evidence convincingly documents that melatonin may have significant utility in combatting neuronal death in Alzheimer's disease (AD); in both experimental studies [64] and a small number of limited clinical reports [65-67] beneficial effects of melatonin have been reported to reduce the pathophysiology caused by two major features of AD, i.e., amyloid-β (Aβ) and neurofibrillary tangle (NFT) toxicities.

One group of culpable agents that mediates the destruction of both neurons and glia in AD is ROS/RNS. The formation of senile plaques, which are composed of Aβ, is a cardinal sign of the AD brain and their excessive deposition leads to oxidation of key molecules in neighboring cells. Of particular interest in this regard is Aβ1-42, since it is the major form of Aβ and readily generates reactive species. That melatonin limits oxidative damage and the associated cellular death of cultured neuroblastoma cells exposed to Aβ has been known for roughly a decade. Furthermore, melatonin is reported to strongly inhibit the spontaneous formation of β-sheets and Aβ-fibrils [64]. The first observation, i.e., the suppression of Aβ toxicity in vitro by melatonin has been repeatedly confirmed [68].

In vivo studies are likewise consistent with melatonin's high efficacy in restricting the ability of Aβ to damage neurons and alter neurophysiology. Thus, the negative neurobehavioral consequences of injection of Aβ25-35 (the portion of the larger Aβ molecule that generates ROS/RNS) directly into the hippocampus of rats was significantly ameliorated by subsequent melatonin treatment [69]. In this case, the neurobehavior of the rats was improved as judged from their performance in Morris water maze, a well known method for testing several forms of memory. Perhaps the most interesting study is one in which melatonin, given in the drinking water, reduced both Aβ deposition in the brain and death of transgenic mice genetically transfected with the human amyloid precursor protein (APP).

NFTs within neurons are also a major feature of AD. Like Aβ, which is an extracellular pathological agent, intracellular NFTs are believed to contribute to the pathology of AD in a number of ways including the generation of free radicals and related reactants. NFTs form as a consequence of the phosphorylation of a cytoskeletal protein, tau. This protein is phosphorylated by a variety of kinases in the brain of individuals suffering with AD. The injection of isoproterenol bilaterally into the hippocampi of rats is known to stimulate protein kinase A (PKA) leading to structural modifications of the tau protein and the formation of intracellular NFTs. The ability of NFTs to form is inhibited in these animals when melatonin is peripherally administered [70]. This action of melatonin is apparent when melatonin is given before or after isoproterenol administration.

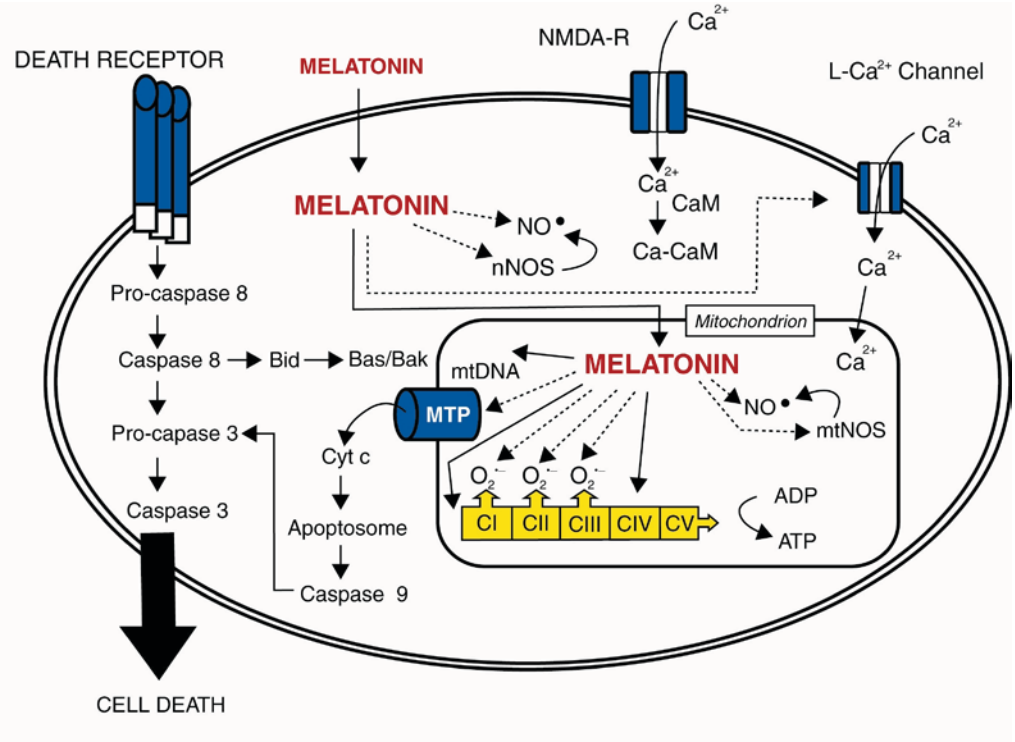
The cholinergic hypothesis of AD speculates that a deficiency of cholinergic neurotransmission likely accounts for some of the cognitive impairments in AD patients. Depressed choline acetyltransferase (ChAT) activity in the frontal cortex and hippocampus are common features of the brain of mice transfected with the human APP. Again, melatonin, given peripherally, promotes ChAT activity in the affected brain areas of APP transgenic mice, reduces DNA damage, limits neuronal apoptosis, and lessens the conspicuous neurobehavioral deficits that normally develop in these animals [71].

Clearly, both the in vitro and in vivo studies, only a few of which are mentioned here, have demonstrated the high efficacy of melatonin in reducing the pathophysiolgical signs characteristic of the human AD brain [72]. These include (i) reduction of Aβ deposition, (ii) limiting the oxidative toxicity of Aβ, (iii) curtailing NFT formation and the associated pathophysiology, and (iv) promoting the formation of acetylcholine which is deficient in CNS models of AD.

Clinical use of melatonin to influence the development or progression of AD in humans has been, unfortunately, limited. Whereas both the number of reports and the number of patients included has been small, the outcomes of the studies suggest melatonin may have utility in reducing the severity of symptoms in AD patients [65-67,73,74]. It is imperative that these preliminary studies be followed up with more complete trials on the use of melatonin in combatting this highly devastating condition.

After AD, PD is the next most common neurodegenerative condition in aged individuals and it affects nearly 2% of individuals over the age of 65 years. A major neuropathological feature of the disease is a persistent loss of dopaminergic neurons in the pars compacta of the substantia nigra. These degenerative changes result in a major reduction in brain dopamine levels which are manifested clinically as defects in motor function, cognitive decline and psychological depression. In addition to the loss of dopaminergic neurons, the brain develops what are referred to as Lewy bodies and neural cytoplasmic inclusions that are composed predominately of fibrillar α-synuclein. Biochemical examination of the brain of PD patients suggest that, either directly or indirectly, ROS/

Figure 3. **Cellular and mitochondrial actions of melatonin which protect against free radical-mediated molecular destruction and cell death. These actions can be furthered examined in a recent review of Leon et al. [44].**



RNS are causative agents in the morphological damage.

The most frequently used animal model of PD is their treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This is also a highly valuable and realistic model since MPTP, even before it was known for including PD-like signs in animals, was shown to cause PD-like symptoms in humans. After its ingestion or administration, MPTP rapidly passes through the blood-brain barrier where it is taken up by glial cells where it is metabolized to 1-methyl-4-phenyl-pyridinium (MPP^+) by monoamine oxidase. MPP^+, after its release by glial cells, utilizes the dopamine transporter to enter dopaminergic cells where it enters synaptosomal vesicles and mitochondria. In the latter organelle, MPP^+ disrupts oxidative phosphorylation by inhibiting Complex I of the mitochondrial electron transport chain. The escape of electrons in the mitochondria elevates damaging free radical generation which contributes to the death of the neurons.

The second most common PD model is the treatment of animals with the neurotoxin 6-hydroxydopamine (6-OHDA). Giving this drug promotes especially the loss of dopaminergic neurons in the substantia nigra of the midbrain. In the cytosol of these neurons, 6-OHDA generates reactive metabolites of oxygen which inactivate a variety of macromolecules; the resultant molecular damage leads to cellular loss and eventually signs of neurodegeneration.

With the discovery of melatonin and its metabolites as aggressive free radical scavengers and indirect antioxidants, the indoleamine was quickly and frequently tested for its ability to reduce the neural toxicity of MPTP and abate the resulting parkinsonian-like signs [65]. Melatonin, in vivo, was found to reduce lipid and DNA damage to neurons inflicted by MPTP. Furthermore, it reduced the inhibitory effect of MPTP on mitochondrial Complex I. Some of the most compelling data documenting the protective actions of melatonin against MPTP comes from a chronic study in which animals were treated with what was considered a low dose of MPTP with or without concomitant melatonin treatment. After 35 days of treatment, immunoreactive tyrosine hydroxylase (TH) activity had virtually disappeared from the striatum and substantia nigra of the animals given MPTP alone. Conversely, animals given melatonin in combination with MPTP had TH levels indistinguishable from those in control animals. The same relationships held when the surviving dopaminergic neurons were counted [75].

In every study in which melatonin has been tested against MPTP/MPP^+ toxicity, it has proven effective in ameliorating the morphological changes and neurobehavioral deficits that normally accompany PD. Also, in these studies [65,68,75] the protective effects of melatonin were surmised to be related to the antioxidant properties of this molecule; this conclusion was frequently inferred from the fact that free radical damage is a significant component of neurological damage in the PD brain and, typically, the degree of neural oxidative stress was reduced after melatonin had been administered.

As with MPTP/MPP^+ toxicity, melatonin is likewise effective in limiting the negative actions of 6-OHDA in vitro and in vivo. Given that the cellular toxicity of 6-OHDA is mediated by elevated generation of free radicals and related

reactants, again, melatonin's protective actions were presumed to be linked to its multi-faceted antioxidative properties and to its ability to preserve the functional integrity of the electron transport chain in the mitochondria [44,76-78] (*Fig. 3*).

Amyotrophic lateral sclerosis (ALS), also known colloquially as Lou Gehrig's disease, is a rapidly progressive neurological disease that is inevitably fatal. This condition is associated with degeneration of upper and lower motoneurons resulting in the loss of voluntary control of muscles. When the muscles of the diaphragm degenerate due to the loss of motoneuron control, the subjects lose the ability to breathe. Although several potential mechanisms have been suggested to explain the mechanisms of the destruction of neurons in the spinal cord and brain, free radical damage seems to contribute to the process. Because of this, it has been surmised that the antioxidant properties of melatonin may be beneficial in this condition; there has been only one test of the theory.

In a preliminary study, it was reported that providing melatonin orally to ALS patients for a one year period slightly improved or delayed the progression of the disease. This finding supports a more complete study of the potential use of melatonin in ameliorating the symptoms of ALS. Since high doses of melatonin (30-60 mg, slow release) were given daily, one major goal of the study was to determine the safety of melatonin in these patients. The authors concluded that melatonin was well tolerated and no side effects were uncovered during the one year treatment period [79].

As with the previously discussed disorders, cerebrovascular accidents including stroke, progressively increase in frequency with advancing age. This is frequently associated with a number of other diseases that often become manifested with age, i.e., hypertension, atherosclerosis, etc., which cause deteriorative changes in the walls of blood vessels. Neural ischemia occurs locally (focal ischemia) when a single blood vessel is involved or the ischemic event can involve the entire brain (global ischemia), e.g., during the transient discontinued beating of the heart. When the brain is reperfused with oxygenated blood after a period of anoxia/hypoxia, the oxygenated blood leads to the production of additional active metabolites of oxygen which exaggerates the free radical damage that is caused by ischemia [80].

The most frequently used model to test the ability of melatonin to curtail free radical damage that occurs of a result of I/R is transitory middle cerebral artery (MCA) occlusion. Numerous studies of this type have been performed in animals with the duration of ischemia and reperfusion varying according to the investigators selected protocol. Also, in these studies the treatment paradigms with melatonin included the injection of the indoleamine just prior to or at the time of ischemia onset, at the onset of reperfusion, or at several intervals after reperfusion onset. Regardless of the melatonin injection paradigm used, it proved effective in reducing the neural damage that results as a consequence of I/R [80-84].

The most common endpoints that were measured and improved when melatonin was given to MCA-occluded rats were (i), infarct volume, (ii), edema, (iii) number of apoptotic neurons, (iv), level of lipid peroxidation, and (v), neurological deficits. Interestingly, although most often pharmacological levels of melatonin were found to limit the neural damage due to I/R, pinealectomy, which causes only a relative melatonin deficiency, was found to exaggerate neural damage after a transitory interruption of the blood supply to the brain and its subsequent reoxygenation. This implies that not only pharmacological concentrations, but physiological levels of melatonin also have some capability of arresting neural damage that occurs during anoxia and reoxygenation.

Based on the results of studies in models of AD, PD, ALS and I/R, melatonin may play a significant role in the "weak" version of the free radical theory of aging. Thus, by reducing the severity of these conditions melatonin could indirectly prolong life.

CONCLUSIONS

This brief report considers the role of melatonin in the "strong" and "weak" (or the direct or indirect) versions of the free radical theory of aging. With regards to the latter version, the review examines in particular the role of melatonin in forestalling brain degenerative changes which often increase in frequency later in life. Unquestionably, aging is generally not considered a good thing and surely nothing will prevent the terminal event, i.e., death. On the other hand, melatonin may have some utility in deferring degenerative conditions that are related to the excessive generation of free radicals. Based on the experimental data that have accumulated and considering its lack of toxicity and that it is much less expensive than prescription drugs, its use should be considered as a potential agent to improve the quality of life in a rapidly aging population and it may possibly secondarily influence life span.

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