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Melatonin, Longevity and Health in the Aged: An Assessment*

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This brief review considers the potential role of melatonin in the processes of aging, the prolongation of life span and health in the aged. Studies completed to date generally suggest that exogenously administered melatonin may serve to extend life span in invertebrates, but evidence supporting this conclusion in mammals is less compelling. Thus, any conclusion regarding a role for melatonin in extending normal longevity, particularly in mammals, would be premature. With regard to deferring the signs of chemically-induced neurodegenerative conditions in experimental animals, the data are remarkably strong and there is a modicum of evidence that in humans with debilitating diseases melatonin may have some beneficial actions. Indeed, this should be one focus of future research since as the number of elderly increases in the population, the frequency of costly age-related diseases will become increasingly burdensome to both the patient and to society as a whole.

Keywords: Melatonin; Age-related diseases; Longevity; Oxidative stress; Aging

Aging is a complex, and undoubtedly, multifaceted process which influences all living organisms. Thus, not surprisingly there are a gamut of theories to explain organismal deterioration over time and numerous approaches have been used to forestall the consequences of this insidious process. Although the theories to explain aging are multiple, most are overlapping in their concepts and are not mutually exclusive. Many of the theories may be at least partially valid.

The diversity of deteriorative processes that account for the functional decline in the elderly probably also explains why it has been difficult to significantly alter the rate of deterioration with a single agent. A variety of factors and/or supplements have been given in an attempt to postpone the anatomical and physiological decline and to impede the onset of pathophysiological states with only partial success. On the other hand, however, these endeavors have not totally or universally failed, particularly with regard to impeding the development of specific disease processes. This leaves open the expectation that we may yet uncover a molecule or group of molecules that may significantly diminish the rate of functional decline or the onset of senescence.

One process that has yielded success in a variety of organisms is the limitation of food intake, i.e. food or calorie restriction.[1] However impractical this treatment may be for the human population, particularly in the economically developed countries, it has been informative in terms of what it has taught us of the processes of senescence. Among many benefits afforded by food restriction has been the retarded production of free radicals associated with delayed signs of aging in these animals along with a prolongation of median and maximal life span. Certainly, free radicals and specifically the gradual but persistent accumulation of free radical-mutilated molecules during life have been widely espoused as being consequential in the aging process.[2]

This brief review summarizes the experimental data that have accumulated relative to the potential

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role of melatonin (*N*-acetyl-5-methoxytryptamine) in deferring aging and/or age-related diseases. Melatonin is an endogenously-produced and an exogenously-acquired indoleamine which has at least hypothetical benefits for aging and for the aged.[3] It relates to the above discussion by virtue of its ability to subvert molecular damage under conditions where free radicals and related reactants are involved. While in the context of such a brief review all data related to the issue cannot be discussed or cited, consulting the acknowledged publications will lead the reader to additional supplemental information that either supports or detracts from melatonin's image as a beneficial agent during aging.

THE NATURE OF MELATONIN

Melatonin was discovered over 40 years ago when it was chemically characterized after its isolation from bovine pineal tissue.[4] The pineal gland of mammals is a neuroectodermal organ situated at the posterodorsal aspect of the third ventricle of the brain. In all mammals including man, it is an end organ of the visual system with the prevailing light–dark cycle, via the eyes, controlling the synthesis and secretion of the indoleamine.[5] Thus, in all species, regardless of their activity pattern, i.e. whether they are diurnal, nocturnal or crepuscular, pineal melatonin is produced primarily during the dark phase of the light–dark cycle. As a consequence, melatonin has been characterized as the "chemical expression of darkness". Melatonin, once produced in the pineal gland, rapidly diffuses into the pervasive capillary bed[5] in the organ as well as into the cerebrospinal fluid of the third ventricle.[6] Surgical removal of the pineal gland in mammals prevents the nighttime rise in circulating melatonin levels but it does not totally deplete the concentrations of this indoleamine in the blood. Likewise, during the day when pineal melatonin production is nearly at a standstill, melatonin is still detectable in the blood of mammals. Typically, daytime concentrations of melatonin in the blood are 5–15 pg/ml serum while at night these values range up to 200 pg/ml.[5]

Once in the blood, melatonin has access to every cell in the organism. There are, in fact, no morphophysiological barriers to melatonin; for example, it readily crosses the blood–brain barrier,[7] the blood–testes barrier[5] and the placenta.[8]

Of particular importance is that melatonin is not in equilibrium in the organism. Thus, its concentration in bodily fluids other than the blood often greatly exceeds levels in the circulation. As examples, melatonin levels in the ovarian follicular fluid,[9] in the bile[10] and in the third ventricular CSF,[6] are significantly higher than simultaneous levels in the blood. Indeed, in the bile and CSF they are several orders of magnitude higher. Likewise, within subcellular compartments melatonin is seemingly not uniformly distributed and, again, the levels may significantly exceed blood concentrations;[7,11] this is an area of research that deserves further exploration. A frequent error that is made by individuals judging the ability of melatonin to function in the reduction of oxidative damage is assuming melatonin levels throughout the body are equivalent to those in the circulation. Quite to the contrary, values can differ by orders of magnitude when they are examined and other fluid and tissue levels of the indoleamine often greatly exceed those in the peripheral circulation.

Perhaps melatonin was initially assumed to be associated with aging because there is an incessant reduction in circulating melatonin throughout life due to the gradual failure of the melatonin synthesizing machinery in the pineal gland.[12] As a result, in the frail elderly melatonin levels are typically a fraction of those in younger individuals. Of interest, however, is that in healthy elderly the melatonin levels are often better preserved than those in individuals in a feeble condition. Although this correlation provides no definitive conclusions regarding any association between melatonin and the aging state, the relationship is of interest to the gerontologist.

Initially proposed as a hormone due to its ability especially to synchronize seasonal breeding in photoperiodic species,[13] hundreds of reports in the last decade have unequivocally documented the ability of melatonin to curtail molecular destruction due to free radicals and associated reactants. In many cases, melatonin's detoxification of toxic reactants are receptor-independent.[14] Receptor-mediated actions are a requisite feature that characterizes a hormone. This, coupled with melatonin's presence in edible foodstuffs,[15] and therefore its ingestion in the diet, render the term hormone inadequate for this indoleamine.

By definition, a hormone conventionally is a molecule produced in an endocrine gland of a multicellular organism that is then released after which it travels to a distant target organ to exert receptor-mediated actions. Melatonin's discovery in unicellular organisms,[16] in the strictest sense questions the use of the term hormone to describe melatonin in these species. It has been proposed that melatonin, a phylogenetically ancient molecule, probably evolved coincident with the large increase in molecular oxygen (O_2) in the environment, and its initial function was to protect organisms from the by-products of O_2 metabolism, i.e. to function as an antioxidant. In multicellular organisms, melatonin has acquired some autocrine, paracrine and hormonal functions as well. The vast literature regarding the ability of melatonin to protect against semi-reduced oxygen species as well as nitrogen-based

TABLE I Some of the reports that have compared the relative efficacies of melatonin (Mel), vitamin C (Vit C) and E (Vit E), in reducing free radical damage that was a result of a variety of toxins/processes *in vivo*

Species	Toxin/process	Mel (μmol/kg)	Vit E (μmol/kg)	Vit C (μmol/kg)	Ratio Vit/mel	Damaged organs or tissues (measured product)	Protective effects			Reference
							Mel	Vit E	Vit C	
Rat	Phosphine	43		170	4.0	Brain (MDA + 4HDA)	−27%		−6%	[20]
						Brain (8-OHdG)	−44%		−17%	
						Liver (MDA + 4HDA)	−21%		−21%	
						Liver (8-OHdG)	−33%		−22%	
Rat	Extra hepatic bile duct ligation	2	35		17.5	Plasma (MDA)	−63%	−19%		[21]
						Liver (MDA)	−49%	−12%		
Rat	Chlorpyrifos-ethyl	43	349	+1136	34.5	Erythrocytes (MDA)	Reduced equal %			[22]
Rat	$KBrO_3$	172*	3488*		20.0	Kidney (8-OHdG)	−25%	−25%		[23]
Mouse	Ethanol	172*	523*		3.0	Liver (mtDNA)	Reduced equal %			[24]
						Heart (mtDNA)	Reduced equal %			
						Brain (mtDNA)	Reduced equal %			
Mouse	Doxorubicin	21	580		28.0	Heart (MDA)	−35%	−26%		[25]
						Long Term Survival	14/20†	12/20†		
Guinea pig	Ischemia-reperfusion	43	373		8.7	Retina (MDA)	−54%	−45%		[26]

The amount of each agent is expressed as the molecular concentration. In most cases, melatonin was more effective in reducing oxidative damage compared to either vitamin, even when concentrations of melatonin were much lower. *Accumulated dose. †Number of surviving animals per total number of animals.

reactants should be consulted as to melatonin's efficacy in reducing molecular damage.[14,17–19]

The low concentration argument has often been used to dispel the notion that melatonin could function as a significant antioxidant. To reiterate, however, melatonin levels within tissues and cells may be much greater than in the blood. It is also essential that any free radial scavenger/antioxidant be at the site where highly reactive free radicals, because of their fleeting half-life, are generated. Positioning of antioxidants relative to free radial production is critical to their efficacy in combating oxidative damage. This explains why vitamin E, for example, is not particularly protective of nuclear DNA *in vivo*. When melatonin has been compared to the antioxidant prowess of either vitamin C or E *in vivo*, it has frequently been far superior (Table I). Also, *in vitro* data indicate that melatonin synergizes with the antioxidant vitamins to reduce molecular damage.[27]

Another consideration that should be taken into account when melatonin's antioxidant activity is evaluated, is its ability to stimulate important antioxidative enzymes, i.e. glutathione peroxidase, glutathione reductase and catalase.[28] Interestingly, the promotion of the activities of these enzymes is likely receptor-mediated. Thus, melatonin seems to possess both receptor-independent and receptor-mediated processes to limit free radical destruction of essential macromolecules. Additionally, melatonin increases the concentration of an important intracellular antioxidant, glutathione, by stimulating its rate-limiting enzyme, γ-glutamylcysteine synthase.[29] Finally, melatonin may act at the mitochondrial level to increase the efficiency of oxidative phosphorylation thereby reducing electron leakage and lowering free radical generation.[30]

A final factor relates to what is referred to as the cascade reaction of melatonin in terms of free radical detoxification. The products that are formed when melatonin interacts with oxygen-based reactants, i.e. N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK)[31] and cyclic 3-hydroxymelatonin [unpublished observations], are likewise highly effective in protecting against oxidative damage. Furthermore, the chief enzymatic hepatic metabolite of melatonin, i.e. 6-hydroxymelatonin, is equivalent to melatonin itself in protecting against free radical damage.[32] The ability of these second generational molecules to function in the detoxification of free radicals increases the effective concentrations of melatonin as an antioxidant.

It is presumably these composite actions of melatonin that afford it the ability to be effective in protecting against free radical damage. The literature is certainly replete with publications showing that, especially *in vivo*, melatonin is in fact highly efficacious in reducing molecular damage when

free radicals are the attacking culprits and, as indicated in Table I, its efficacy in doing so often exceeds that of vitamins C and E.

MELATONIN: RELATION TO AGING AND AGE-RELATED DISEASES

If there is validity to the widely-accepted view that aging and some age-related diseases are, at least in part, a consequence of accumulated free radical damage, the so-called free radical theory of aging,[2] then it is justifiable to consider a potential role for melatonin in these processes. Speculations regarding this issue surfaced when the observations were made that endogenous melatonin production diminishes in the aged.[12] This alone, however, is not the only evidence linking melatonin with the vagaries of aging and the progression of age-related diseases, particularly neurodegenerative diseases.[33]

There have been several studies which have directly examined melatonin's effect on longevity. In one of the first investigations of this type, the claim was made that melatonin, given in the drinking water nightly to either BALB/c or New Zealand Black (NZB) female mice, postponed aging and prolonged life.[37] These observations are, however, compromised by the fact that the daytime administration of melatonin to NZB mice was inconsequential in altering the duration of survival. This report further claimed that when the pineal gland was transplanted into the thymus of mice it also prolonged their survival. This observation has been justifiably questioned since the transplanted pineal gland, because of the loss of its sympathetic innervation, is by all measures functionally inept. Hence, the reported observations as to how pineal transplants allegedly influenced the life span is left without a viable explanation. On the whole, the observations reported by Pierpaoli and Regelson[34] are not widely accepted by the scientific community.

Also using mice (NZB/W), Lenz and colleagues[35] found that the daily injection of 100 μg melatonin beginning at 8 months of age prolonged their life relative to that of placebo-treated controls. In this study, daily melatonin injections were given either in the morning (08:00–10:00 h) or in the afternoon (17:00–19:00 h) with the morning melatonin injections having a slightly greater life prolonging effect. A mechanism to explain the prolongation of life span by melatonin was not uncovered in this study.

In another more recent investigation which again used female mice (CBA) that were given melatonin in their drinking water (for 5 days per week) beginning when the animals were 6 months of age, a statistically significant prolongation of survival was noted.[36] Until the mice were 22 months of age, no change in survival rate was apparent. Thereafter, a pronounced reduction in the frequency of mortality in the melatonin-treated animals was observed. In the animals receiving melatonin, the number that reached the age of 24 months increased 4.5-fold in comparison with the controls. The mean life and maximal life spans were also extended by the melatonin treatment (Table II). A seemingly unusual observation was a slight increase in frequency of tumor growth in the melatonin-treated mice. While this would normally be expected in longer surviving animals, it is nevertheless unusual since it is well documented that a variety of tumor types are normally inhibited by melatonin.[37]

The fruit fly *Drosophila melanogaster* (Oregon wild strain) has also been used to test the effect of melatonin on life span. Studies using species such as the fruit fly have an advantage over the use of mammals in as much as typically large numbers are included in each group; that was also the case in the report of Bonilla and co-workers[38] where 560 flies were included in the life span studies. When melatonin was added to the nutritional medium (100 μg/ml), the maximal life span was increased from 61.2 days in the controls to 81.5 days in the melatonin-fed flies, an enhancement of 33.2% (Fig. 1). Likewise, melatonin prolonged the median life span (13.5%) and the onset of 90% mortality (19.3%). It is conceivable that had Bonilla *et al.*[38] selected a strain of fruit flies with a normally shorter life span, the life prolonging effects of melatonin may have been greater. On the basis of additional studies, this group surmised that the antioxidative actions of melatonin were responsible for the increased survival time of *D. melanogaster*. This was deduced from the findings that melatonin also increased the resistance of flies to paraquat as well as to thermal stress (ambient temperature of 36°C), two treatments that promote free radical generation. In the survival tests, however, they never actually documented reduced oxidative damage in the melatonin-fed flies.

The observations on fruit fly survival after melatonin treatment are seemingly consistent with studies that used melatonin-deficient rats,[39] although the purpose of the latter investigation was

TABLE II Life span parameters of female CBA mice given melatonin (2 mg/l) in their drinking water 5 days each week beginning when they were 6 months of age until their natural death

Parameters	Control	Melatonin
Mean life span	685 ± 9.2	722 ± 12.6* (5.4%)
Mean life span (last 10 survivors)	738 ± 1.1	793 ± 18.6* (7.4%)
Median life span	705	747 (5.9%)
Maximal life span	740	867 (17.1%)

There were 50 mice per group. *$p < 0.05$ versus controls; values in parentheses are percentage differences from controls; variance = SE; from Ref. [35].

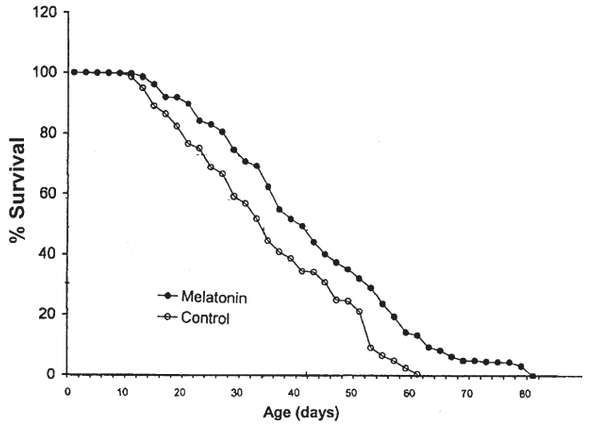

FIGURE 1 Kaplan–Meier survival curves of control and melatonin-fed *D. melanogaster*. Each point is the mean of four life span studies in which a total of 560 flies were used.

not to test the effect of melatonin on the duration of survival. In the study in question, 14 male Sprague–Dawley rats were pinealectomized and an additional 14 rats were sham-operated when they were 2 months of age. By the time these animals reached 25 months of age, 8 of the 14 (57%) pinealectomized rats were still surviving while 13 of 14 (93%) pineal-intact animals were alive. Thus, although it was not statistically verified because of the small numbers of animals, it appeared that rats lacking their pineal gland (and therefore being melatonin-deficient) were dying at an earlier age. At this point all remaining rats were killed and tissues were analyzed for the degree of oxidative damage. This revealed that the amount of lipid peroxidation products, protein carbonyls and a damaged DNA product (8-hydroxy-2′-deoxyguanosine) in tissues of the pinealectomized rats were significantly elevated over those in the rats that had a pineal gland. The implication of these findings is that animals that live their life in a melatonin-deficient state (due to pinealectomy) suffer from increased oxidative damage and die at a younger age. This study, however, did not prove a role for melatonin in as much as the indole was not given to an additional group of rats lacking their pineal gland to determine whether it would have in fact reduced the oxidative damage. It could be argued that some other change induced by pinealectomy (e.g. circadian rhythm disruption), may have accounted for the reduction in survival and the augmented oxidative damage.

The presumption that melatonin may increase life span has also been tested in the ciliated protozoan *Paramecium tetraurelia*. According to Thomas and Smith-Sonneborn,[40] the addition of melatonin (10 mg/l) to the nutrient medium (bacterized Cerophyl) increased mean and maximal clonal life spans of paramecia by 20.8–24.2% and 14.8–24.0%, respectively. Since others have reported that vitamins C and E have a similar effect in promoting clonal life span of paramecia, the authors feel that melatonin's beneficial actions may relate to its antioxidant properties.

It is well documented that melatonin protects against molecular mutilation induced by a variety of free radical-generating drugs and other situations where elevated oxidative stress is involved.[41] This being the case, it is not unexpected that the indoleamine would also prolong survival of animals exposed to toxins or to situations that induce a high oxidative state. As an example, melatonin reduced the fatality rate in mice infected with *Schistosoma mansoni*, an infestation associated with elevated oxidative stress.[42] Likewise, Lissoni[43] summarized observations on 1440 terminally-ill cancer patients receiving either supportive care (718 patients) or supportive care plus melatonin (722 patients). In this case, melatonin treatment stabilized the disease or caused a modest reduction of tumor size in some patients while significantly increasing one-year survival as well as life quality. The antitumor activity of melatonin is in part receptor-mediated and is well documented as an inhibitory agent for some tumor types.[37]

Considering the role of free radicals in neurodegenerative diseases,[44] which are common in the aged, it would be anticipated that melatonin may possibly defer the progression of these diseases and, thereby, prolong survival. There is, in fact, an extensive literature showing that melatonin alleviates the severity of neuronal loss and dysfunction in experimental models of neurodegeneration; likewise, there is a modicum of evidence that melatonin may also be beneficial in Alzheimer's patients in terms of reducing the symptoms of this devastating disease.[33,45]

CONCLUDING REMARKS

While it is premature to conclude, on the basis of the data summarized herein, that melatonin treatment promotes increased survival in mammals, there is evidence to suggest this may be the case. Deficiencies in regard to the studies that have observed an effect of melatonin on life prolongation include some inconsistent results, small numbers of animals with insufficient controls and the inclusion of animals that were not specific pathogen-free and barrier-maintained. Additional research is obviously required to clarify whether melatonin has an influence on natural life span. In contrast, the evidence suggesting that melatonin may promote survival in animals/individuals with an abbreviated life expectancy because of disease or toxin exposure is generally more consistent and future research should be directed to this possibility.

If, in fact, melatonin proves beneficial in increasing survival and/or reducing the severity of debilitating diseases, the mechanisms remain to be clarified. Possible explanations include its ability to reduce oxidative stress,[14,17–19,28] to strengthen the immune system,[46] to improve mitochondrial function,[47] and to synchronize circadian rhythms.[48] Other potential actions, however, should also be explored.

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