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Melatonin: Its Role in Limiting Macromolecular Toxicity Due to Partially Reduced Oxygen Metabolites

Introduction

Melatonin, originally thought to be exclusively of pineal origin (Wurtman and Axelrod, 1965), is now known to derive from other tissues as well. Besides the pineal gland, a few of the tissues that may produce melatonin include the retina (Iuvone, 1986), lacrimal gland (Mhatre et al., 1988), bone marrow (Tan et al., 1999a; Conti et al., 2000), and possibly other cells as well (Kvetnoy, 1999; Stefulj et al., 2001).

Some of melatonin's functions have been well defined although the specific mechanisms by which it executes its effect have remained elusive. Unquestionably, the circadian production of melatonin influences seasonal reproduction in photoperiodic species (Reiter, 1980), retinal physiology (Dubocovich, 1986), tumor cell proliferation (Blask, 2001), immune system function (Guerrero et al., 1997a; Maestroni et al., 1997), circadian rhythms (Lewy and Sack, 1993), and possibly sleep (Jan et al., 2000) among others. In general,

these actions of melatonin seem to be mediated by specific membrane receptors for the indole (Masson-Pevet et al., 1996; Shiu et al., 1996) although an action at the nuclear level (Carlberg, 2000) is also feasible. Likewise, other potential actions of melatonin may become manifested after it binds to calmodulin (Pozo et al., 1997).

In addition to this there is one action of melatonin which is independent of any receptor or binding site. Thus, melatonin directly neutralizes free radicals and other reactants by electron donation (Hardeland et al., 1993; Poeggeler et al., 1994; Reiter et al., 1996). This action and associated functions of melatonin is the subject of this brief review. For more details on the mechanisms of melatonin as an antioxidant and free radical scavenger, the reader could consult more extensive summaries of recently published data in this field (Tan et al., 2000a; Reiter et al., 2000a, 2001a).

Melatonin Concentrations in Fluids and Subcellular Compartments

An open question regarding the potential importance of melatonin as a free radical scavenger and antioxidant is its concentrations within organisms. Usually when melatonin levels are estimated in subcellular compartments the concentrations are based on what is known about the levels of this indole in the blood which, by the standards of other antioxidants, are low. Thus, it has been commonplace to conclude that while melatonin is a highly effective pharmacological antioxidant, it may not be so at physiological concentrations. The data, however, are not fully supportive of this assumption.

Firstly, to presume that blood levels of melatonin are indicative of intracellular concentrations of the indole may be an error. It would generally seem unwise to make predictions about tissue concentrations of melatonin based exclusively of what would be expected from circulating values. Two recent discoveries on the levels of melatonin in other bodily fluids especially emphasize this. Skinner and Malpoux (1999) and Tan and colleagues (1999b) recently reported that melatonin concentrations in the third ventricular cerebrospinal fluid (Fig. 1) and bile (Fig. 2), respectively, are

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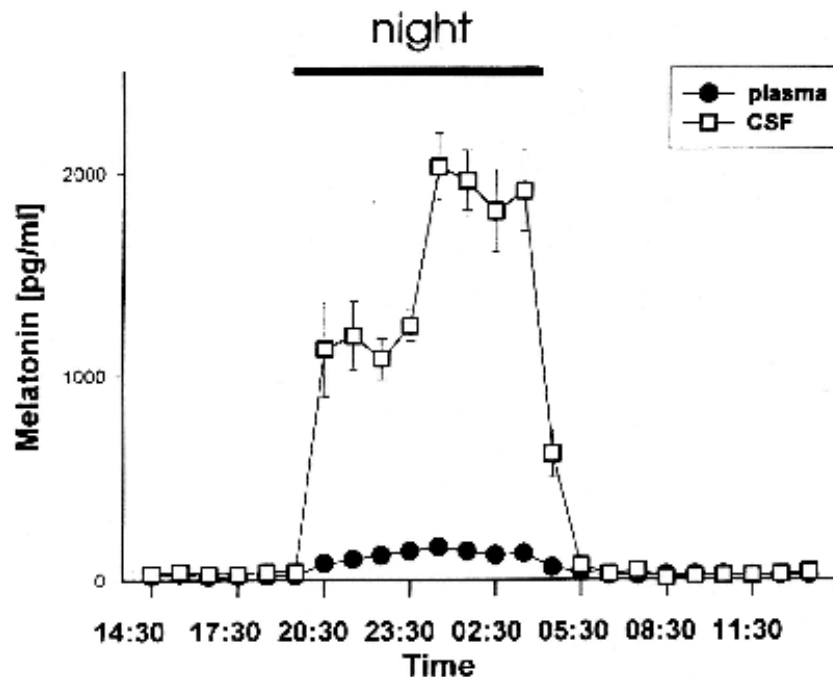


Fig. 1: Melatonin rhythms in the cerebrospinal fluid (CSF) and plasma of sheep over a 24 hour period. The sheep, like other animals including the human, exhibits a nocturnal rise in plasma melatonin with values at night reaching 50–150 pg/ml as shown here. In contrast, CSF levels of melatonin at night are several orders of magnitude higher than daytime levels, reaching values of about 2000 pg/ml. Clearly, plasma and CSF melatonin concentrations are not in equilibrium (Skinner and Malpoux, 1999).

several orders of magnitude greater than those in the blood. Likewise, we also reported that in bone marrow cells the levels of melatonin are far greater than in the blood (Tan et al., 1999a). So clearly bodily fluid and tissue concentrations, at least at these sites, are not contingent upon levels in the peripheral circulation. Even 8 months after surgical removal of the pineal gland, bone marrow concentrations of melatonin remain at very high levels (Tan et al., 1999a).

There are also experimental data which support the idea that physiological concentrations of melatonin are relevant in terms of antioxidative protection. For example, Benot and colleagues (1998, 1999) in both rats and humans have shown that blood levels of melatonin and the total antioxidative capacity of that fluid run in parallel. Thus, the nocturnal physiological rise in melatonin is accompanied by a concurrent increase in the capacity of the fluid to neutralize free radicals. Also, when humans are exposed to light at night to suppress the rise in melatonin, the total antioxidant capacity of the blood is attenuated. The strong parallelism between blood concentration of mela-

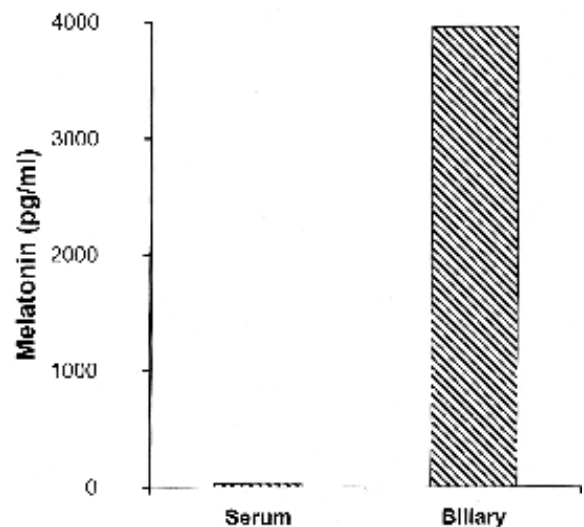


Fig. 2: Rabbit serum and bile levels of melatonin in fluids collected simultaneously. As in the CSF (Fig. 1), there is no correlation between the melatonin levels in these two fluids. Subcellular organelles, in those where measurements have been made, also have melatonin concentrations in excess of those measured in the blood.

tonin and its antioxidant capacity was also confirmed by Albarran and co-workers (2001) in an avian species.

Cardinali and colleagues (2001) have recently considered the issue of subcellular concentrations of melatonin. Accordingly, they point out that, as a lipophilic substance, melatonin's entrance into cellular membranes under both physiological and pharmacological conditions may be much higher than circulating levels of this indole. Already two decades ago radioimmunoassayable levels of melatonin in the rat hypothalamus were reported to be 1–4 pg/mg tissue (Pang and Brown, 1983; Catala et al., 1987). When HPLC was employed these concentrations were found to be as high as 10 pg/mg in rat hypothalamus at night (Cardinali et al., 2001). From these data it is obvious that melatonin levels in the tissues in question may well be within the high nanomolar range assuming a homogenous distribution of melatonin in various subcellular compartments. These values could be substantially higher if melatonin is differentially distributed within subcellular compartments. In fact, an unequal distribution of melatonin in intracellular organelles is likely. Based on a number of studies (Menendez-Pelaez and Reiter, 1993; Menendez-Pelaez et al., 1993; Tan et al., 1999a; Martin et al., 2000), preliminary findings indicate highest melatonin levels are found in the nucleus and in mitochondria. In these organelles melatonin levels could easily be in the micromolar range or higher (Cardinali et al., 2001). This is also emphasized by the fact that in a unicellular organism (*Gonyaulax polyedra*), concentrations of melatonin intracellularly can be normally induced to be in the millimolar range (Antolin et al., 1997).

Clearly, physiological levels of melatonin can vary widely depending on the organism, the cell type and the specific subcellular organelle. Obviously, to make judgements about physiological intracellular concentrations of melatonin based on levels of this indole in the blood are misleading. Additional investigations in this area are needed to clarify as to what constitutes a physiological level of melatonin.

That physiological melatonin concentrations are adequate to reduce oxidative damage to a variety of macromolecules in a number of tissues also is documented. In several reports pinealectomy was employed to reduce endogenous levels of the indole. In these studies, oxidative damage in the brain was exaggerated after either transient focal ischemia or following excitotoxicity (Manev et al., 1996; Kilic et al., 1999) compared to the level of damage in the brain of rats experiencing the same oxidative insult but possessing an intact pineal gland. Likewise, rats living the bulk of their life with a relative melatonin deficiency due to early pineal removal, also exhibited increased damage to polyunsaturated fatty acids, proteins and DNA in many organs (Reiter et al., 1999). Finally, the exposure of rats to constant light, a procedure which suppresses physiological levels of melatonin, increases tissue concentrations of damaged lipid products (malondialdehyde and conjugated dienes); these changes were reversed when constant light-exposed rats were supplemented with melatonin (Baydas et al., 2001). Thus, reductions in physiological concentrations of melatonin exaggerate oxidative damage indicating that, at these levels, melatonin is relevant in reducing oxidative damage.

Antioxidant Properties of Melatonin: Mechanisms

After ground state oxygen is inhaled, its reduced metabolites can become highly toxic. This, coupled with oxygen's obvious benefit for the survival of aerobic organisms, is referred to as the "oxygen paradox". Thus, while oxygen is required for survival of aerobes, its metabolic products may slowly bludgeon cells to death over the course of a lifetime.

An estimated 2–4% of the O₂ inhaled is actually converted to reactive, toxic intermediates. The pathways by which these reactants are formed

are summarized in figure 3. In addition to the oxygen-based reactants, some of these toxic agents are nitrogen-based. Members of both groups of metabolites can be highly destructive of macromolecules (Trush and Kensler, 1991; Kehrer, 1993; Halliwell, 1994).

That melatonin effectively neutralizes a variety of oxygen and nitrogen-based reactants is no longer questioned (Reiter, 1998, 2000; Reiter et al., 1997). In many cases even the mechanisms and the resulting products have been identified

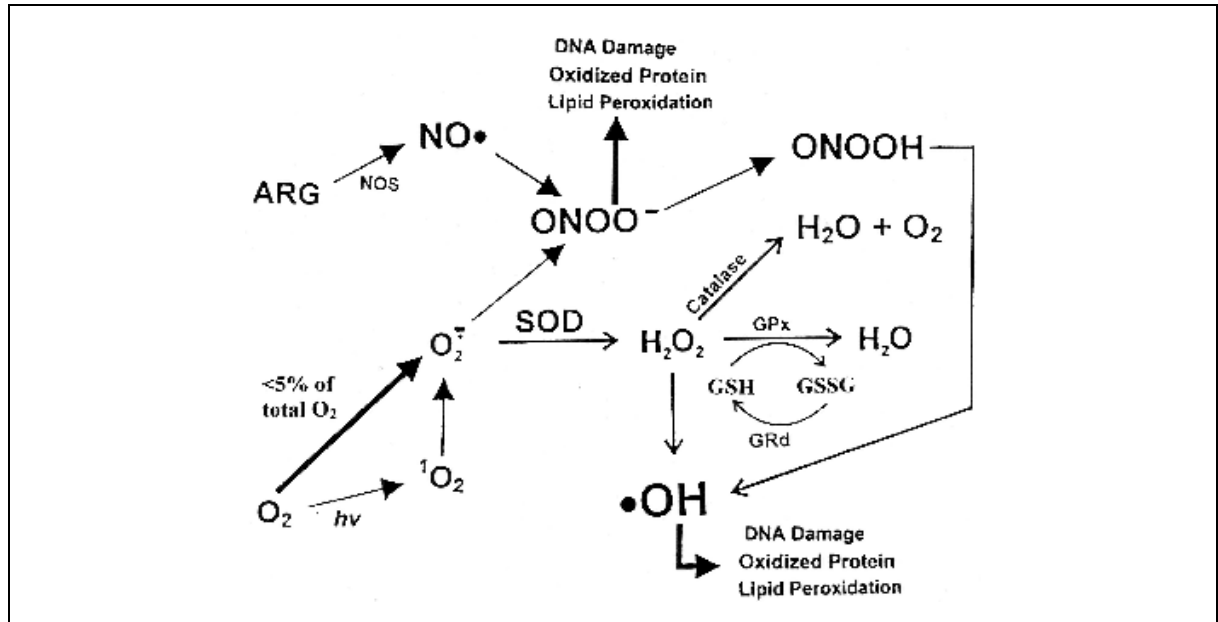


Fig. 3: Roughly 2–4% of the inspired ground state oxygen (O_2) inhaled by aerobes is chemically reduced to highly reactive metabolites which damage macromolecules. Also shown is the formation of nitrogen-based reactants. The two most toxic agents are generally believed to be the hydroxyl radical ($\cdot OH$) and the peroxynitrite anion ($ONOO^-$). These agents indiscriminately damage any molecule in the vicinity of where they are produced. Also shown is the enzymatic metabolism of hydrogen peroxide (H_2O_2) by catalase and glutathione peroxidase (GPx) as well as the recycling of oxidized glutathione (GSSG) back to its reduced form (GSH) by glutathione reductase (GRd).

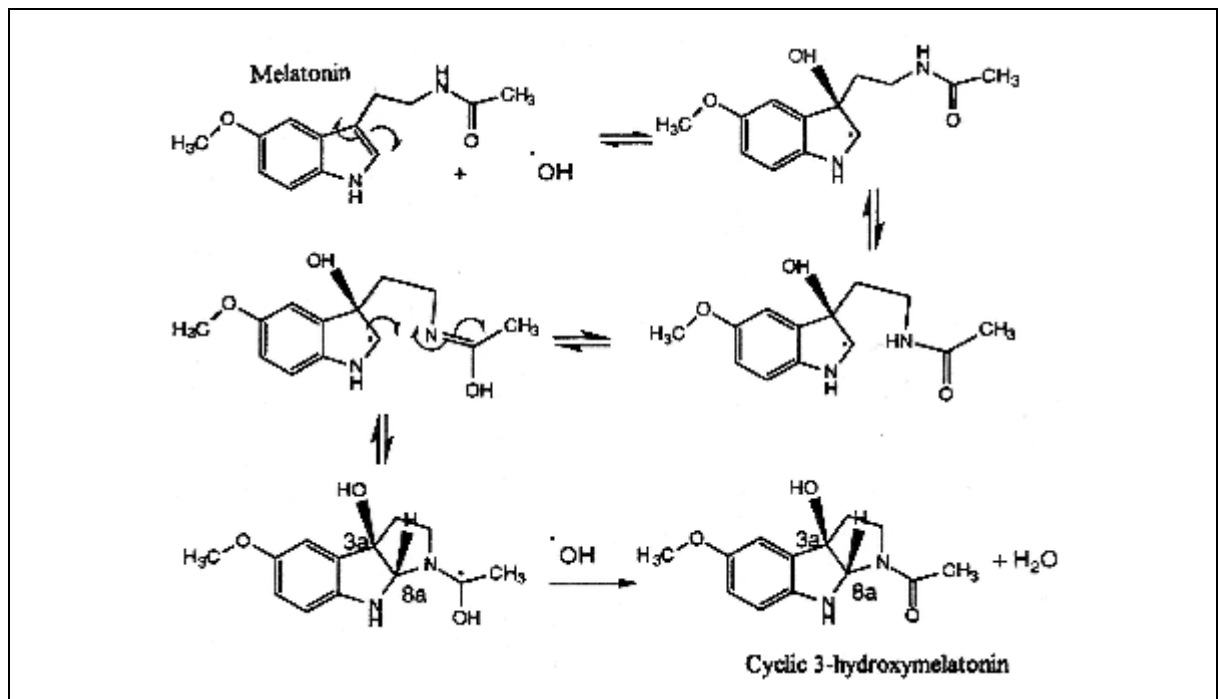


Fig. 4: Scavenging of hydroxyl radical ($\cdot OH$) by melatonin. Initially, melatonin donates an electron to neutralize a $\cdot OH$; in doing so melatonin itself becomes a radical, i.e., melatonyl radical. This undergoes some molecular rearrangement and eventually scavenges a second $\cdot OH$ to form the metabolite cyclic 3-hydroxymelatonin which is passed in the urine. Thus, urinary cyclic 3-hydroxymelatonin is a footprint of the number of $\cdot OH$ melatonin has scavenged. Its urinary concentration varies with the level of oxidative stress in the animal.

(Reiter et al., 2000a, 2001a; Tan et al., 2000a). Thus, when melatonin neutralizes the highly toxic hydroxyl radical ($\bullet\text{OH}$) the resulting product is cyclic 3-hydroxymelatonin (cyclic 3-OHM) (Fig. 4) (Tan et al., 1998a). In this process it should also be noted that each molecule of melatonin actually scavenges two $\bullet\text{OH}$. After its production, cyclic 3-OHM is excreted in the urine where it serves as an index of the number of $\bullet\text{OH}$ that melatonin has scavenged in vivo.

A wide variety of methodologies have been used to document the $\bullet\text{OH}$ scavenging activity of melatonin (Tan et al., 1993; Matuszek et al., 1997; Susa et al., 1997; Stasica et al., 1998; Tan et al., 1998a; Bandyopadhyay et al., 2000). Additionally, in these studies the $\bullet\text{OH}$ was generated using methods that have been widely employed in free radical research (Tan et al., 1993; Poeggeler et al., 1996; Pähkla et al., 1998; Khaldy et al., 2000; Brömme et al., 2000; Ebel et al., 2000). The find-

ing that melatonin neutralizes the $\bullet\text{OH}$ is important since it is estimated that in excess of 50 % of the molecular damage cells sustain may be a specific consequence of the toxicity of the $\bullet\text{OH}$ (Fig. 5). Furthermore, $\bullet\text{OH}$ is not enzymatically removed from cells. On the other hand, there are numerous agents capable of detoxifying the highly reactive $\bullet\text{OH}$.

Even a more efficient means of reducing $\bullet\text{OH}$ -mediated damage would be to prevent its formation by removing its precursor, H_2O_2 (Fig. 3). Here melatonin functions in several ways. Unlike the $\bullet\text{OH}$, H_2O_2 is normally metabolized to non-toxic products by two different enzyme systems, i.e., the glutathione peroxidases (GPx) and catalase (CAT) (Fig. 3). Data have been published showing that melatonin promotes the activities of both these enzymes, thereby reducing steady state levels of H_2O_2 and consequentially lowering $\bullet\text{OH}$ generation (Barlow-Walden et al., 1995; Montilla

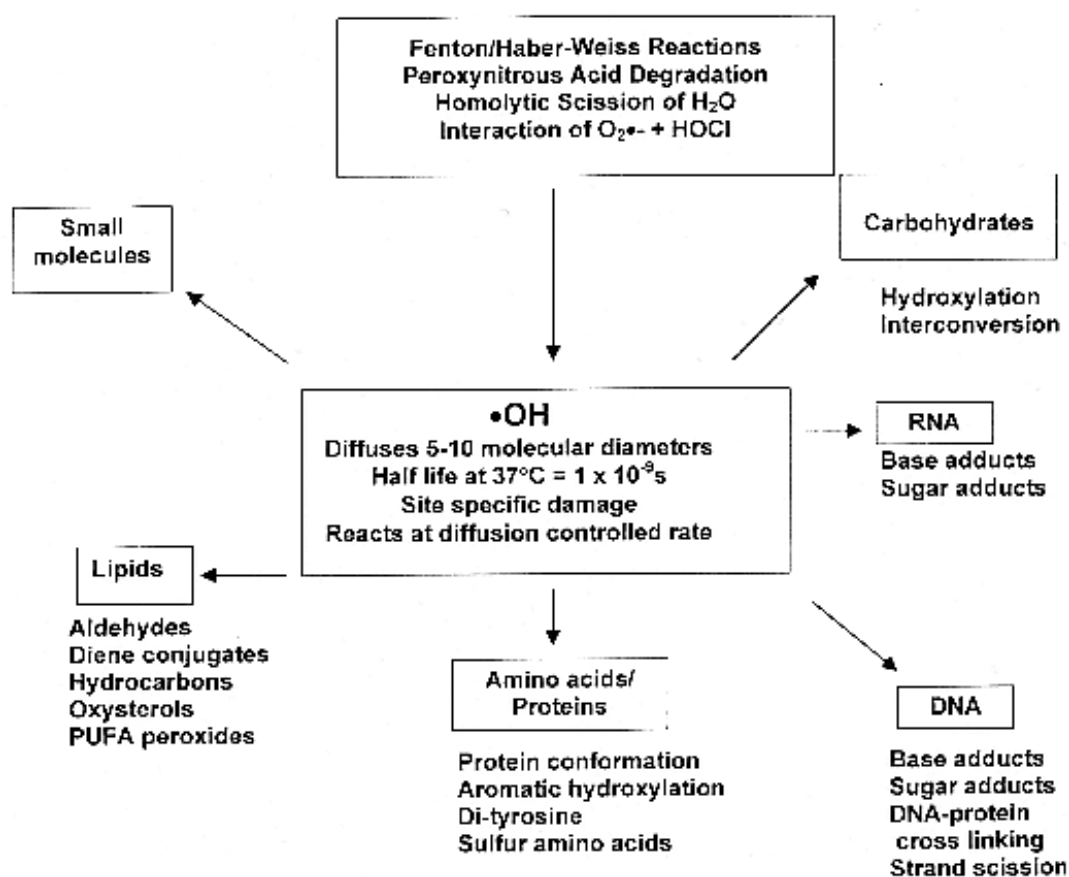


Fig. 5: Formation and damaging reactions of the hydroxyl radical ($\bullet\text{OH}$). As shown at the top/center, the $\bullet\text{OH}$ is generated via a number of reactions. Also, it damages any molecule in the vicinity of where it is generated resulting in the formation of a number of products that are routinely measured as indices of oxidative stress.

et al., 1997a, 1997b; Pablos et al., 1997a, 1998). In some cases melatonin appears to directly stimulate the activities of GPx and CAT while in others it may be a matter of preventing the inhibitory effect of oxidative stress on these enzymes. Since these enzymes are large proteins they could be readily inactivated by free radicals; the antioxidant melatonin would be expected to protect against this damage and therefore against enzyme inactivation.

Besides its modulatory effects on H_2O_2 -metabolizing enzymes, recent data shows that melatonin also directly detoxifies this reactive intermediate (Tan et al., 2000b). In a cell free system, we found that melatonin scavenges H_2O_2 in a concentration-dependent manner. With the aid of C^{14} - and H^1 -nuclear magnetic resonance, we also identified the product that is formed as a result of the direct interaction of melatonin with H_2O_2 ; this metabolite is *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine (AFMK) (Fig. 6). When monitored in solution, AFMK accumulates in a pattern inverse to the loss of H_2O_2 . Thus, while melatonin promotes the activities of the major H_2O_2 -metabolizing enzymes, it also directly scavenges this

reactive intermediate. In combination with melatonin's direct scavenging effect on $\bullet OH$, this combination of actions would presumably function in significantly lowering the steady state concentrations of the highly reactive and ubiquitously destructive $\bullet OH$.

The fate of AFMK, once it is formed, has been investigated and it was found also to be a highly effective antioxidant (D.X. Tan, L.C. Manchester, R.J. Reiter, unpublished observations). Indeed, it may be a better free radical scavenger than melatonin itself. Using cyclic voltametry, AFMK was shown to be capable of donating two electrons (unlike most free radical scavengers which typically donate a single electron). In vitro AFMK reduced chromium(III)-induced DNA damage to purified calf thymus DNA, inhibited lipid peroxidation in hepatic homogenates caused by H_2O_2 plus Fe^{2+} , and prevented the death of hippocampal neurons incubated with H_2O_2 . Collectively, the findings indicate that AFMK, like melatonin itself, is remarkably protective against oxidative stress.

The observations regarding melatonin and its metabolite, AFMK, indicate there is a cascade of reactions which makes the parent molecule, melatonin, highly effective in reducing oxidative injury. Not only is melatonin itself a radical scavenger, but also at least one of its metabolites is as well.

Accumulated evidence suggests that the $ONOO^-$ may be equivalent to the $\bullet OH$ in terms of its oxidative toxicity (Beckman and Koppenol, 1996). This may be due to the inherent reactivity of $ONOO^-$ or because it degrades into the $\bullet OH$ (Pryor and Squadrito, 1995). That melatonin scavenges the $ONOO^-$ has also been shown by two groups. Accordingly, both Zhang and colleagues (1998, 1999) and Blanchard and co-workers (2000) showed that melatonin neutralizes both $ONOO^-$ and peroxynitrous acid ($ONOOH$) (Fig. 3); furthermore, they identified several potential products that are a result of these interactions.

As pointed out above, $ONOO^-$ is a result of the coupling of the superoxide anion ($O_2^{\bullet -}$) with nitric oxide ($NO\bullet$) (Fig. 3) (Pryor and Squadrito, 1995). Indeed, the toxicity of $NO\bullet$ is commonly attributed to $ONOO^-$ rather than to $NO\bullet$ itself. Thus, any agent which reduces $NO\bullet$ concentrations would also lower the levels of $ONOO^-$ and thereby diminish the resulting oxidative injury.

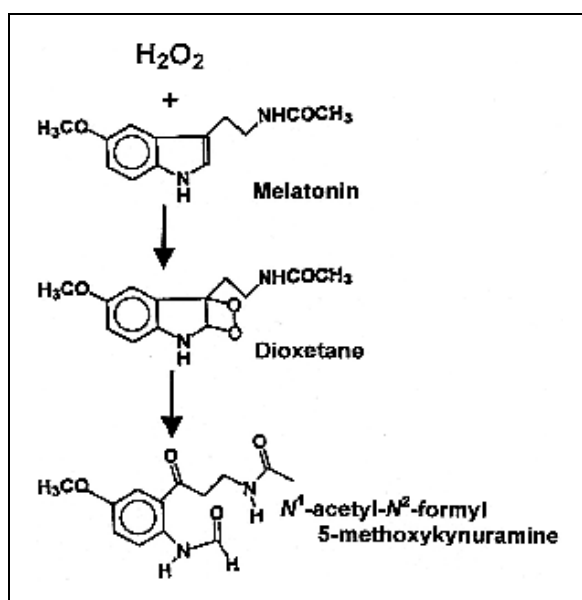


Fig. 6: Scavenging of hydrogen peroxide (H_2O_2) by melatonin to form *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine (AFMK). AFMK is also a highly effective free radical scavenger. Thus, melatonin and AFMK function as a cascade of reactions to neutralized free radicals. One proposed intermediate in this reaction is dioxetane.

Melatonin reduces $\text{NO}\bullet$ levels in at least two ways. Firstly, it inhibits its synthesis from arginine by limiting the activity of its rate-limiting enzyme, inducible nitric oxide synthase (NOS) (Pozo et al., 1997). The proposed mechanism of melatonin relates to its binding to calmodulin inasmuch as NOS is calmodulin dependent. Additionally, as first shown by Noda and co-workers (1999) and subsequently confirmed by Turjanski et al. (2000, 2001), melatonin also directly scavenges $\text{NO}\bullet$. The direct scavenging of $\text{NO}\bullet$ by melatonin has been invoked as an explanation for the mild vasoconstrictor activity of the indole in the human umbilical artery (Okatani et al., 2001a).

The most obvious controversy regarding melatonin's scavenging effects is in reference to the peroxy radical ($\text{ROO}\bullet$). Initially espoused as a more potent $\text{ROO}\bullet$ scavenger than vitamin E (Pieri et al., 1995), later studies seriously questioned these observations (Antunes et al., 1999). If in fact melatonin is a more potent scavenger of the $\text{ROO}\bullet$ than is vitamin E, one would also expect it to have a greater inhibitory effect on lipid

peroxidation. Tests have generally shown that in homogenates of tissues, vitamin E more reliably reduces lipid breakdown than does melatonin (Escames et al., 1997). In vivo, however, the opposite may be the case. The greater efficacy of melatonin in vivo presumably relates to its broad spectrum antioxidative actions and its more copious uptake compared to vitamin E. Interesting, using purified proteins in vitro where damage was believed to be a result of $\text{ROO}\bullet$, melatonin and vitamin E were roughly equally protective against oxidative damage (Salvi et al., 2001). Clearly, these seemingly contrasting effects cannot readily be explained on the basis of what is currently known of the $\text{ROO}\bullet$ scavenging activity of melatonin.

There are many more reports relating to melatonin's direct and indirect scavenging actions than are presented here (Pablos et al., 1997b; Urata et al., 1999). Space limitations preclude a discussion of all reports pertaining to this issue, but they can be located by consulting other review articles (Hardeland et al., 1993; Reiter et al., 2000a, 2001a, 2001b; Tan et al., 2000a).

Verification of Melatonin's Antioxidative Actions

Despite the fact that melatonin was discovered to function as an antioxidant only a decade ago, the literature related to its protective actions against free radical damage is already voluminous (Fig. 7). Remarkably, melatonin has been shown to attenuate oxidative destruction initiated by a vast array of toxins and processes and in a number

of disease models. As mentioned above, this brief review does not permit a discussion of all the published reports that have investigated melatonin's protective effects; thus, only selected subjects will be highlighted here.

Because of their short half-lives, it is difficult to estimate direct free radical generation. Rather it is

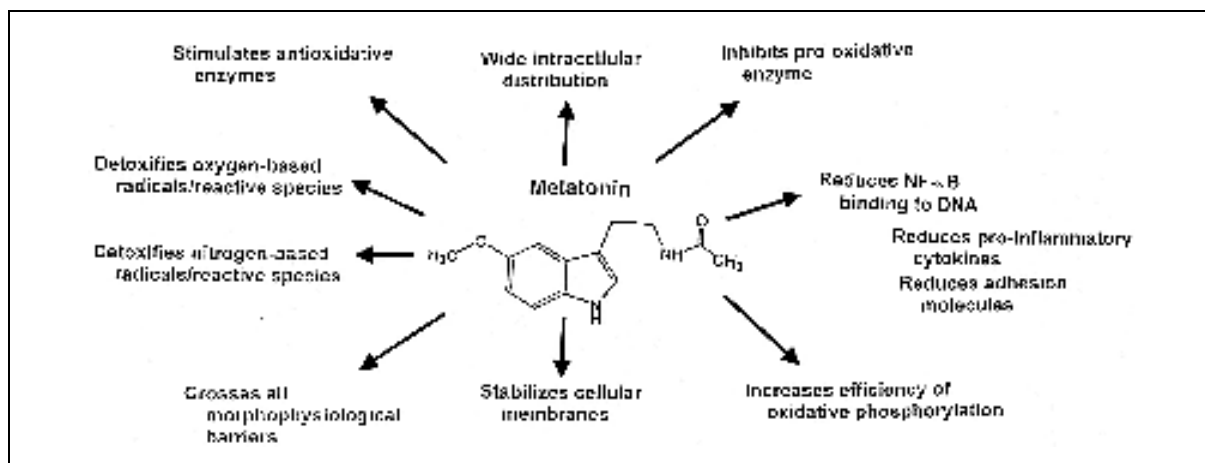


Fig. 7: Features that make melatonin a ubiquitously acting direct free radical scavenger and indirect antioxidant. Many of these functions of melatonin are discussed in the text.

common to measure the damage that is a consequence of these molecular brigands. The endpoints that are most typically measured include levels of lipid peroxidation products, damaged proteins and altered DNA. Both in *in vitro* and *in vivo* studies, melatonin has been shown effective in reducing oxidative destruction to each of these macromolecules (Reiter et al., 1997; Reiter, 1999). These observations are also consistent with the widespread distribution of melatonin within the cell since the majority of the polyunsaturated fatty acids (PUFA) are located in the cellular membranes, DNA is restricted to the nucleus and mitochondria and proteins are distributed throughout the cell. Hence, if the protection of each of these macromolecules is a consequence of direct free radical scavenging, melatonin must be located at all these sites in order to resist the bulk of the damage that would normally occur. Furthermore, melatonin would likely have to be there in greater concentrations than are normally found in the blood.

PUFA are especially susceptible to oxidative damage and the assays for measuring the resulting products are in widespread use. Thus, this is one of the most common parameters used to estimate free radical damage. Membrane lipids are oxidized under situations such as toxin exposure, hyperbaric hyperoxia, ischemia-reperfusion, physical stress and as a consequence of drug administration. In all of these situations, melatonin administration prevents the breakdown of lipids (Pablos et al., 1997b; Ebel et al., 2000; Cuzzocrea et al., 2001; Hara et al., 2001; Wakatsuki et al., 2001).

Similarly, in all situations in which proteins were damaged by oxygen or nitrogen-based reactants concurrent melatonin treatment overcame the destructive processes (Reiter, 1999; Kim et al., 2000; Ohta et al., 2000; Salvi et al., 2001). In the study of Kim and co-workers (2000) melatonin was found to be as effective as glutathione in reducing induced protein carbonyl formation. Finally, melatonin's protective effects against DNA damage have been well documented under a variety of conditions wherein free radicals would otherwise destroy the genetic material (Uz et al., 1996; Wakatsuki et al., 1999; Ohta et al., 2000; Qi et al., 2000a, 2000b). In at least one of these conditions, melatonin was more protective of oxidant-induced damage to DNA than was either vitamins C or E (Fig. 8) (Qi et al., 2000b).

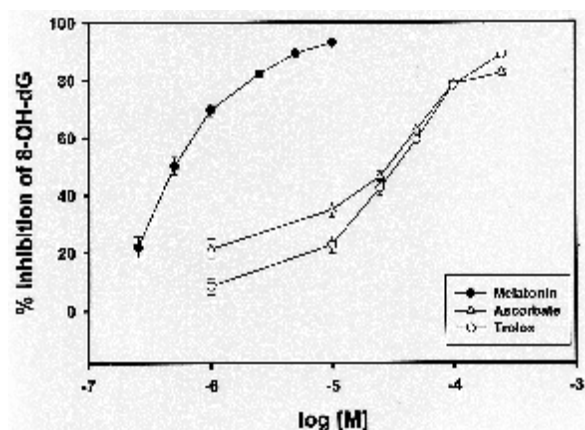


Fig. 8: Concentrations of melatonin, vitamin C and vitamin E (Trolox) required to inhibit damage to purified calf thymus DNA induced by 500 μ M chromium and H_2O_2 . By calculating the IC_{50} (concentration of each that would reduce oxidative damage by 50%), it was shown that melatonin was roughly 60 times more effective than either of the vitamins in reducing DNA damage as indicated by levels of 8-hydroxy-2-deoxyguanosine (8-OHdG).

As lipids in membranes become oxidized, the membranes become increasingly rigid (less fluid). Membrane fluidity is not uncommonly used as an index of the degree of PUFA breakdown. Using this endpoint, Garcia and colleagues (1997, 1998, 1999) have shown, in a series of experiments, that the increased rigidity of microsomal membranes due to the oxidation of the inherent lipids is also reversed by the addition of melatonin to the mixture. Changes in membrane fluidity and the percentage of lipids oxidized do not always run in parallel and some evidence suggests that melatonin may assist in maintaining optimal membrane fluidity even in the absence of oxidative stress. This may relate to the ability of melatonin to position itself between the polar heads of the membrane lipid moieties (Tesoriere et al., 1999).

Free radicals and related agents have been proposed as potential causative factors in a large number of diseases. As a consequence, many reports have appeared in which melatonin has been tested as a preventive agent in models of these diseases. Some of the more important models in which the efficacy of melatonin has been examined as an ameliorative agent have involved the central nervous system (Reiter, 1998). Thus, the indole has been found to protect against the toxicity of amyloid- β peptide, a major constituent of

Alzheimer's disease (Pappolla et al., 1997, 1998, 2000; Bozner et al., 1997; Olivieri et al., 2000), against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (a chemical used to destroy dopaminergic neurons which simulates Parkinson's disease) (Acuña-Castroviejo et al., 1997; Iacovitti et al., 1997; Mayo et al., 1998) and against quinolinic acid (which causes signs similar to those of Huntington's disease) (Southgate et al., 1998; Behan et al., 1999; Cabrera et al., 2000). The outcome of these studies has prompted the use of melatonin in treating individuals with Alzheimer's disease (Brusco et al., 1998).

Other clinically-relevant neurological models in which melatonin's beneficial actions have been examined include ischemia/reperfusion (stroke) injury and glutamate toxicity. In both of these conditions, free radicals and other reactants are considered a major component of the resulting cytotoxicity. Both physiological and pharmacological levels of melatonin were shown to modify the degree of neural damage sustained following a period of middle cerebral artery occlusion and reperfusion. Thus, Manev et al. (1996), Kilic and colleagues (1999) and Joo and co-workers (1998) all showed that pinealectomy, a procedure that reduces but does not totally deplete endogenous melatonin, aggravated stroke damage (in terms of infarct volume and neuronal loss). Substituting melatonin by injecting it during the period of reperfusion lowered infarct volume by up to 40%. The ability of exogenously-administered pharmacological concentrations of melatonin, usually given prior to the induction of the ischemic period or simultaneous with the onset of reperfusion, to limit the resulting deficits in pineal intact animals also has been tested and found effective (Cho et al., 1997; Ling et al., 1999; Cuzzocrea et al., 2000a). Several important issues remain to be resolved, however. Thus, studies are lacking in terms of dose-response relationships, definition of the "window of efficacy" for melatonin treatment and whether melatonin, given chronically in advance of the hypoxia/reoxygenation episode, would be prophylactically beneficial in reducing the severity and extent of neural lesions. While each of the studies summarized above used the rat as the experimental subject, one group also used the Mongolian gerbil (*Meriones unguiculatus*) (Guerrero et al., 1997b); this species is frequently employed for such studies because the cir-

cle of Willis on the base of the brain is incomplete. Thus, in the absence of collateral circulation the forebrain can be deprived totally of oxygenated blood by temporarily constricting the carotid arteries bilaterally. Again, in this species melatonin reduced damage to the brain resulting from temporary interruption of blood flow in the carotid arteries.

In each of these reports, the authors attributed the beneficial effects of melatonin to its free radical scavenging and ancillary activities. Even fetal brain injury after occlusion of the ovarian arteries in pregnant rats has been shown to be reduced when melatonin is administered to the mother (Wakatsuki et al., 1999). Melatonin is known to readily cross the placenta (Okatani et al., 1998).

In addition to its efficacy in reducing brain injury after hypoxia/reoxygenation, melatonin has been used in studies with other organs where blood flow was transiently interrupted. Of particular note is melatonin's ability to reduce the severity of cardiac damage after a period of ischemia followed by reperfusion, i.e., heart attack. Tan and co-workers (1998b) found that melatonin was 30 times more effective than vitamin C in reducing the cardiac arrhythmias associated with interruption and then restoration of blood flow to the heart. Similarly, Lagneux and co-workers (2000) described melatonin to impart "spectacular protection" against both the irregular cardiac contractions and cardiac lesion size after ischemia/reperfusion. Finally, in the retina (Osborne et al., 1998), liver (Fig. 9) (Sewerynek et al., 1996a) and gut (de la Lastra et al., 1997; Cuzzocrea et al., 2000b) melatonin reduced the loss of tissue and the compromised function that followed hypoxia/reoxygenation. In these collective studies many different parameters of cytotoxicity, molecular damage and function were measured; without exception, all benefited when melatonin was administered. As in the brain, no dose-response relationships have been established nor has the "window of efficacy" for melatonin been defined in any of these organs.

There are now numerous published studies where melatonin has been used to combat oxidative damage in other situations. Space limitations preclude a discussion of all these studies in this brief review. Only a few of the additional reports will be mentioned here. Hence, melatonin has been effectively used to negate what is believed to be free

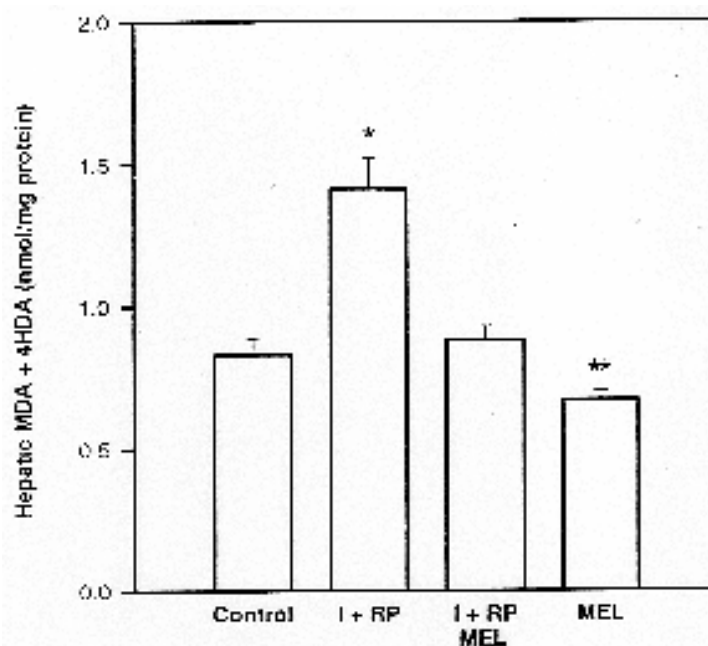


Fig. 9: Lipid peroxidation products [malondialdehyde (MDA) and 4-hydroxyalkenals (4HDA)] in the liver of rats where the organ was subjected to ischemia/reperfusion (I + RP). Melatonin prevented the degradation of lipids in the liver due to ischemia/reperfusion. Also, melatonin lowered basal levels of lipid peroxidation products in the liver of rats not subject to ischemia/reperfusion. * $P < 0.001$ versus all other groups; ** $p < 0.05$ versus controls.

radical toxicity in models of diabetes (Montilla et al., 1998; Ebel et al., 2000; Sailaja Devi et al., 2000), traumatic brain injury (Mesenge et al., 1998), endotoxin administration (Sewerynek et al., 1996a), inflammation (Cuzzocrea et al., 1999; Reiter et al., 2000c), circulatory shock (El-Sokkary et al., 1999), athrogenesis (Pieri et al., 1996; Seeger et al., 1997; Okatani et al., 2001b), porphyrias (Princ et al., 1998; Karbownik et al.,

2000; Qi et al., 2001) and many others. More comprehensive reviews can be consulted for details of these studies (Karbownik and Reiter, 2000; Reiter et al., 2000b, 2000c, 2001b; Reiter, 2001). They uniformly document that melatonin invariably reduces damage associated with these conditions and that the indole has never been shown to aggravate the situation, i.e., to have prooxidative actions.

Concluding Remarks

This brief survey was meant to provide only a sample of the large numbers of studies that have investigated the antioxidative actions of melatonin and the ubiquity of its beneficial effects in experimental models of free radical damage and diseases where oxygen and nitrogen-based reactants are believed to be causative (Fig. 7). The presentation is no where near exhaustive in terms of the citation of relevant papers and, as mentioned above, other sources of information are available.

What is clear from this summary and from the published data is that melatonin may well be ben-

eficial in a number of diseases and clinical situations where antioxidants would otherwise be helpful in reducing molecular and cellular damage. To date, melatonin has only been sparingly investigated in specific diseases. Considering its limited acute and chronic toxicity, however, it is anticipated that it will be more widely tested in humans in the near future and, based on the published reports in animals, there is every expectation that it would be beneficial in a number of clinical situations. Already, preliminary studies have shown melatonin to reduce the incidence of seizures

(Molina-Carballo et al., 1997), to defer the progression of Alzheimer's disease (Brusco et al., 1998) and to lower mortality and oxidative damage in newborn infants associated with septic shock (Gitto et al., 2001) or with asphyxia (Fulia et al., 2001).

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