

# Oxidative damage to nuclear DNA: amelioration by melatonin

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*Submitted:* February 18, 1999

*Accepted:* April 27, 1999

*Key words:* **melatonin; pineal gland; antioxidant**

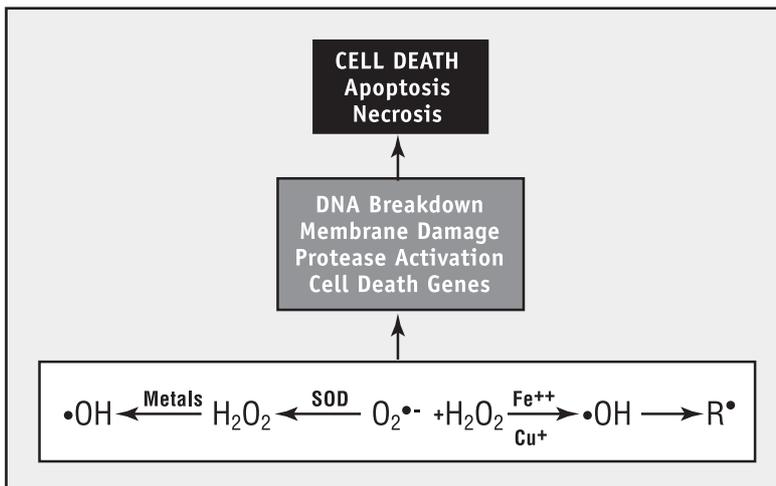
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Neuroendocrinology Letters 1999; **20**:145-150 pii: NEL203499R02 Copyright © Neuroendocrinology Letters 1999

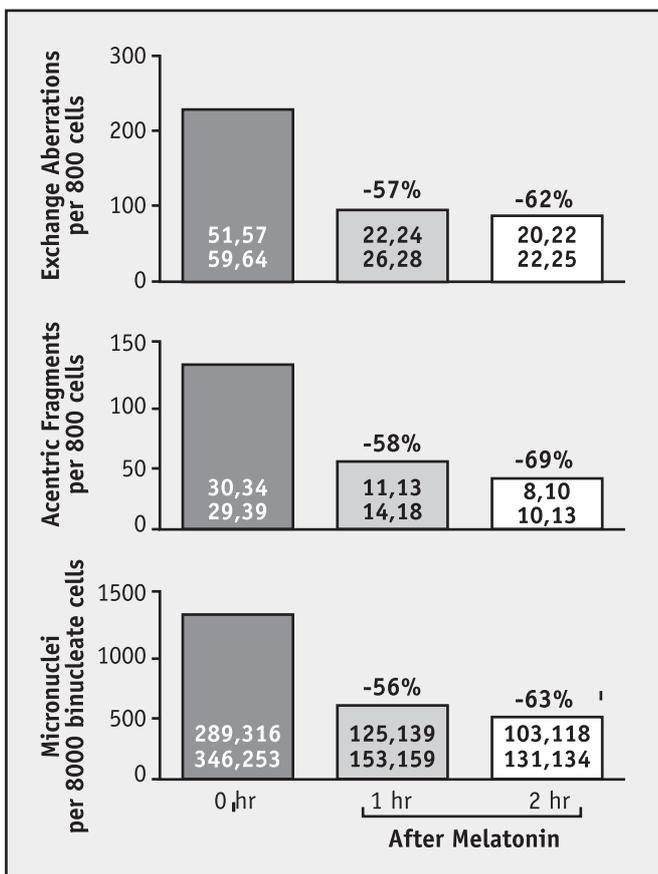
## **Abstract**

The indoleamine melatonin, a product of tryptophan metabolism in the pineal gland, is a free radical scavenger and antioxidant. This brief review summarizes melatonin's ability to protect nuclear DNA from oxidative damage. Using a variety of different cytogenetic, biochemical and molecular biological methods, a number of investigators have demonstrated melatonin's ability to protect DNA from the physical agent ionizing radiation, the herbicide paraquat, the carcinogen safrole, the excitotoxin kainic acid, the heavy metal chromium (VI), and a wide number of mutagens. Since these agents damage DNA because they generate free radicals, the observations are consistent with melatonin being a radical scavenger and antioxidant.





**Fig. 2.** Free radicals generated by the reduction of melatonin are capable of damaging a variety of macromolecules including DNA, as represented here. In addition to potentially causing cell death, cells that contain damaged DNA and survive can become cancerous.



**Fig. 3.** Exchange aberrations, acentric fragments and micronuclei—all signs of damaged genomic material—in the blood of four adult humans. A blood sample was collected before (0 hr) taking melatonin orally and at 1 and 2 hr after consuming melatonin. The lymphocytes from each blood sample were then subjected to 150cGy ionizing radiation. Clearly after melatonin treatment, each index of oxidative damage was suppressed. The percentage reductions are indicated above the bars; within the bars are the four individual values.

[7, 8, 24, 25] and is located in the nucleus [5, 26] becomes relevant to both DNA damage and cancer [27].

Besides damage directly inflicted to DNA by free radicals, there are other mechanisms by which radicals cause destruction of the genome. As already mentioned, lipid peroxides enter the nucleus where they react with Fe(II) to generate the alkoxy radical which attacks DNA [28]. Also, intracellular calcium levels increase as a result of oxidative damage to cellular membranes [29]; calcium then enters the nucleus where it can activate nucleases which cause DNA strand breaks [30].

### Protection of DNA by melatonin

Many of the biological consequences of ionizing radiation are believed to be a result of free radicals generated by this physical agent. While high energy radiation has direct effects on the molecular structure of DNA due to the disruption of chemical bonds, a significant portion of the genomic damage is related to free radicals as shown by the fact that scavengers of these molecular brigands prevent much of the destruction resulting from the exposure of cells or animals to ionizing radiation [31, 32]. Considering the involvement of free radicals in DNA disruption produced by high-energy radiation, it was an obvious model in which to examine the free radical scavenging activity of melatonin.

A variety of cytogenetic procedures was used by Vijayalaxmi and co-workers [33–35] to assess nuclear DNA damage in human blood cells treated with ionizing radiation either alone or in combination with melatonin. The endpoints used by these workers included acentric fragments, micronuclei, sister chromaotid exchanges, and chromosomal aberrations. The counts of these defects were always done on cells for which the treatment was unknown to the person examining the slides. Regardless of the endpoint being evaluated, the augmented genetic damage caused by exposing the cells to ionizing radiation was significantly reduced when melatonin was present in the incubation medium. In one case, melatonin was consumed by individuals and blood samples were drawn when their circulating melatonin levels were highly elevated. This treatment reduced genetic damage in the lymphocytes which were exposed to ionizing radiation after their removal from the individual who consumed the melatonin (Fig. 3) [36, 37]. The implication of the study is that high circulating levels of melatonin could protect humans from ionizing radiation, e.g., routine x-rays, and thereby decrease the incidence of cancer.

These findings are consistent with the presence of melatonin in the nucleus of cells [5, 26] and with its free radical scavenging activities [6, 38, 39]. Indeed, melatonin's antioxidant features are the most likely explanation for the ability of this indole to protect against genetic damage by ionizing radiation although other mechanisms may also be operative [40]. Besides reducing genomic damage which follows exposure to ionizing radiation, melatonin also reduces the mortality of mice which are given lethal doses of high energy radiation. This effect of melatonin also is attributed to its ability to neutralize free radicals and therefore the resulting molecular damage [41].

While antioxidants including vitamins E and C and  $\beta$ -carotene have also been shown to be protective against radiation-induced DNA damage, it is common for these agents to be given in large doses for several days prior to the radiation exposure [42]. In the case of melatonin, it was always given as a single dose just prior to the exposure of cells or animals to high energy radiation. At this point, no studies comparing the relative efficacy of melatonin and the antioxidant vitamins as radioprotectors have been published.

Using the same genetic endpoints employed by Vijayalaxmi and colleagues [33, 34], Melchiorri et al. [43] have shown that melatonin also prevents DNA damage in mice treated with paraquat. Paraquat is a highly toxic herbicide which damages cells via processes involving free radicals [44]. Melchiorri and colleagues [45] had previously found that melatonin reduces other toxic side effects of paraquat as well.

Many chemical carcinogens damage DNA thereby increasing the incidence of cancer; the destructive effects of these agents on the genetic material involve a variety of different mechanisms but in many cases free radicals are involved. In a series of two reports, Tan and colleagues [46, 47] showed that DNA damage caused by the chemical carcinogen safrole was reduced by both physiological and pharmacological levels of melatonin. In these studies DNA adducts were quantified in hepatocytes of rats treated with safrole alone or in combination with melatonin.

Recently, a comprehensive investigation of melatonin's antigenotoxic effects was conducted using the *in vitro* Ames test and the single cell gel electrophoresis assay, i.e., the comet assay [48]. In this case, the ability of melatonin to protect against the mutagenicity of the following twelve agents was tested: 7,12-dimethylbenz(*a*)anthracene, benzo(*a*)pyrene, 2-aminofluorene, 1,2-dimethylhydrazine, bleomycin, cyclophosphamide, 4-nitroquinoline-N-oxide, 2,4,7-trinitro-9-fluorenone, 9-aminoacridine, *N*-nitrosomethylurea, mitomycin C and sodium azide.

While the results show that melatonin protects against DNA damage due to virtually all these agents, there was one unusual finding. Thus, when mitomycin C was used as a mutagen, melatonin exacerbated the responses. This unusual and unexpected finding requires confirmation since there is no reasonable explanation for this result. With that expectation, however, melatonin when evaluated in both the Ames test and with the comet assay proved effective in reducing genetic damage by a wide variety of mutagens. The toxicity of bleomycin, a radiomimetic, was reduced by melatonin consistent with the findings of Vijayalaxmi and colleagues [37, 40] who used ionizing radiation as the DNA damaging agent.

Other agents which induce DNA damage have also been used in combination with melatonin to determine whether the indole has the capability of reducing their toxicity. Kainic acid, an excitotoxin, is well known for its ability to damage DNA in neural tissue, an effect that can be prevented if melatonin is administered in advance of kainic acid administration [49, 50]. In these studies two different methods were used to assess the toxicity to DNA, i.e., dUTP-biotin nick end labeling [49] and the molecular analysis of the levels of 8-hydroxydeoxyguanosine, a damaged DNA product [50].

Heavy metals are known to be genotoxic due to their ability to generate free radicals intracellularly. Susa and co-workers [25] examined melatonin's ability to protect against one such metal, i.e., chromium(VI). Using single strand breaks as an index of DNA damage, it was the conclusion of these workers that melatonin is a potent scavenger of the hydroxyl radical and a powerful protector of DNA from oxidative damage induced by chromium(IV).

Finally, Lai and Singh [51] using the comet assay found that the exposure of rats to extremely low frequency magnetic fields resulted in damage to neural DNA. When they treated the rats with melatonin in advance of their exposure to the magnetic fields, DNA fragmentation was prevented. They interpreted these findings to mean that by mechanisms that are yet to be identified the magnetic fields prolonged the half life of free radicals increasing the likelihood they would interact with DNA. They further assumed that the availability of melatonin in the brain scavenged the radicals, thereby preventing the genomic damage.

There are yet other studies that have shown that melatonin enters the nucleus where it either directly or indirectly protects DNA from oxidative damage. The results of these reports are summarized in a couple of recent reviews [6, 27].

## Final commentary

The studies summarized herein conclusively show that melatonin is capable of protecting nuclear DNA from oxidative challenges induced by a variety of agents. These findings have several implications. Firstly, they illustrate that melatonin *in vivo* is an effective free radical scavenger and antioxidant. Secondly, they demonstrate that exogenously administered or endogenously produced melatonin readily traverses the cell membrane and enters the nucleus. While the bulk of the studies summarized herein were pharmacological in terms of the doses of melatonin administered, there are also studies showing that suppression of endogenous melatonin levels increases oxidative damage when a free radical generating agent or process is introduced [14, 47]. These findings are consistent with the observations that the total antioxidant status of the blood correlates with endogenous melatonin levels (Fig. 4) [52] and that the quantity of melatonin normally produced in the organism is significant in terms of the free radical damage that is sustained on a daily basis.

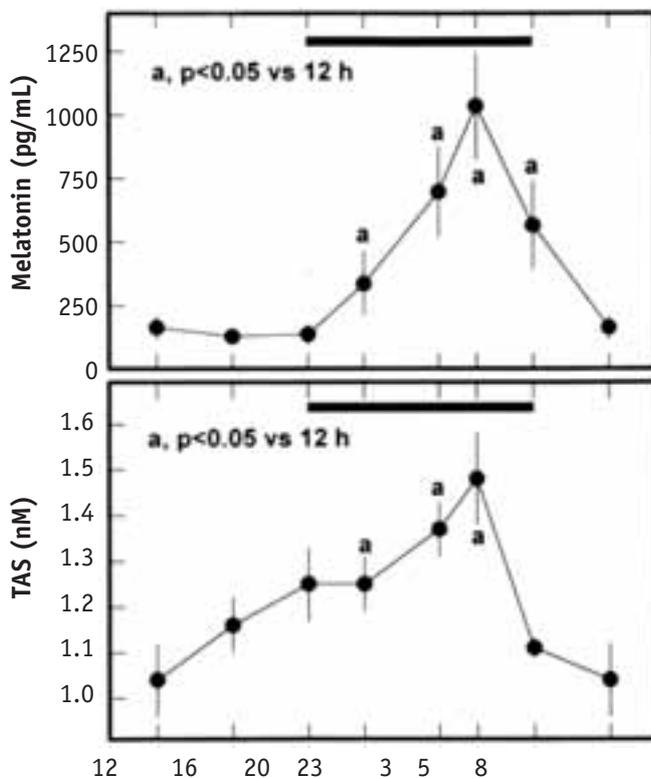
It is generally accepted that the total amount of damage to DNA during the course of a lifetime relates to cancer incidence. Fortunately, DNA dam-

aged by any means has access to a variety of repair mechanisms. While melatonin clearly has the capability of inhibiting the initial damage, to date it has not yet been determined whether melatonin hastens DNA repair processes. In some of the experiments summarized herein this possibly was not excluded and should be tested.

Finally, it does appear that melatonin levels may relate to cancer frequency and growth. Such suggestions have been made in the past [53] and the findings summarized herein are generally supportive of this idea.

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**Fig. 4.** Relationship between the circadian change in blood melatonin concentrations and the total antioxidant status of the blood in rats. As melatonin increases during the daily dark period (represented by the solid black line) so does the capacity of the blood to reduce oxidative damage.

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