Potential anticarcinogenic action of melatonin and other antioxidants mediated by antioxidative mechanisms

Malgorzata Karbownik¹,²
1. Department of Thyroidology, Institute of Endocrinology, Medical University of Lodz, Lodz, Poland
2. Department of Endocrinology, Polish Mother’s Memorial Hospital – Research Institute, Lodz, Poland

Correspondence to: Malgorzata Karbownik, M.D., Ph.D.
Department of Thyroidology, Institute of Endocrinology
Medical University of Lodz
5, Dr. Sterling St., 91-425 Lodz, Poland
Phone/Fax: 48 42 6322594
E-mail: MKarbownik@hotmail.com

Submitted: October 11, 2001
Accepted: November 20, 2001

Key words: melatonin; protection; antioxidants; oxidative stress; carcinogens; DNA; membranes cancer initiation

Abstract
The complex process of carcinogenesis is, to a large extent, due to oxidative stress. Numerous indicators of oxidative damage are enhanced in the result of the action of carcinogens. Several antioxidants protect, with different efficacy, against oxidative abuse, exerted by carcinogens. Recently, melatonin (N-acetyl-5-methoxytryptamine) and some other indoleamines have gained particular meaning in the defense against oxidative stress and, consequently, carcinogenesis. Some antioxidants, like ascorbic acid, play a bivalent role in the antioxidative defense, revealing, under specific conditions, prooxidative effects. Among known antioxidants, melatonin is particularly frequently applied in experimental models of anticarcinogenic action. In the numerous studies, examining several parameters of oxidative damage and using several in vitro and in vivo models, this indoleamine has been shown to protect DNA and cellular membranes from the oxidative abuse caused by carcinogens. When either preventing or decreasing the oxidative damage to macromolecules, melatonin also protects against the initiation of cancer. The protection provided by melatonin and some other antioxidants against cellular damage, due to carcinogens, make them potential therapeutic supplements in the conditions of increased cancer risk.
Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ALA</td>
<td>δ-Aminolevulinic acid</td>
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<tr>
<td>CAT</td>
<td>catalase</td>
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<tr>
<td>Cd</td>
<td>cadmium</td>
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<tr>
<td>Cr</td>
<td>chromium</td>
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<tr>
<td>Cr6+</td>
<td>hexavalent chromium</td>
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<tr>
<td>Cr3+</td>
<td>trivalent chromium</td>
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<tr>
<td>E2</td>
<td>1,3,5(10)-Estratriene-3,17β-diol (17β-estradiol)</td>
</tr>
<tr>
<td>Fe2+</td>
<td>ferrous ion</td>
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<tr>
<td>Fe3+</td>
<td>ferric ion</td>
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<tr>
<td>Fe-NTA</td>
<td>ferric nitritrocitrate</td>
</tr>
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<td>G-</td>
<td>guanosine radical</td>
</tr>
<tr>
<td>5HIAA</td>
<td>5-hydroxy-indole-3-acetic acid</td>
</tr>
<tr>
<td>H2O2</td>
<td>hydrogen peroxide</td>
</tr>
<tr>
<td>IPA</td>
<td>indole-3-propionic acid</td>
</tr>
<tr>
<td>IR</td>
<td>ionizing radiation</td>
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<tr>
<td>KBrO3</td>
<td>potassium bromate</td>
</tr>
<tr>
<td>MDA+4-HDA</td>
<td>malondialdehyde + 4-hydroxyalkenals</td>
</tr>
<tr>
<td>NAS</td>
<td>N-acetylsertotonin</td>
</tr>
<tr>
<td>NOS</td>
<td>nitric oxide synthase</td>
</tr>
<tr>
<td>NO-</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>2-NP</td>
<td>2-nitropropane</td>
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<tr>
<td>O2-</td>
<td>the superoxide anion radical</td>
</tr>
<tr>
<td>iO2</td>
<td>singlet oxygen</td>
</tr>
<tr>
<td>lO2</td>
<td>hydroxyl radical</td>
</tr>
<tr>
<td>ONOO</td>
<td>peroxynitrite anion</td>
</tr>
<tr>
<td>8oxodGuo</td>
<td>8-oxo-2′-deoxyguanosine</td>
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<tr>
<td>PH3</td>
<td>phosphine</td>
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<tr>
<td>PHZ</td>
<td>phenylhydrazine</td>
</tr>
<tr>
<td>pinoline</td>
<td>6-methoxy-1,2,3,4-tetrahydro-β-carboline</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
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</table>

1. Introduction

1.1. Oxidative stress as one of the mechanisms of the process carcinogenesis

There is a balance between the production and detoxification of reactive oxygen species (ROS) under physiological conditions [1, 2]. Any internal or external pathological factor, carcinogens included, may disrupt this balance, leading to conditions referred to as oxidative stress; indeed, oxidative stress plays a significant role in the pathogenesis of cancer [3]. Oxidative stress participates in all the steps of carcinogenesis; at the first step, an initiation, free radical damage different molecules – DNA, lipids, and protein, leading directly or indirectly to mutations and, consequently, to cancer initiation [4].

The products of oxidative damage to DNA, lipid, and protein constitute markers of oxidative damage [5] but, at the same time, they may contribute per se to DNA damage and, in consequence, to cancer development [6–9]. For instance, 8-oxo-2′-deoxyguanosine (8oxodGuo), a product of DNA damage, is highly mutagenic [6, 7]. Numerous byproducts of lipid peroxidation damage DNA via different mechanisms [7, 8]. The oxidative damage to proteins may result in changes of enzyme activities and of some properties of membranes, like permeability, fluidity, signaling pathway, etc. [9].

Both endogenous and exogenous antioxidants can prevent the formation of early metabolites of the damage to macromolecules and, in this way, protect against cancer.

1.2. Potential mechanisms of the anticarcinogenic action of melatonin and other antioxidants

Melatonin (N-acetyl-5-methoxytryptamine) is one of the well documented antioxidants and free radical scavengers [10–16]. Much evidence has already been accumulated for the preventive action of melatonin against cancer [13, 17–22]. The anticarcinogenic action of melatonin is related mainly to its antioxidative and free radical scavenging ability.

Melatonin is widely distributed throughout the organism, being present in all cellular compartments, with its highest concentration in the nucleus [23]; this compartment contains DNA, being directly affected by the process of carcinogenesis. An evidence is accumulated that, besides the pineal gland, melatonin is produced in some other organs, as well [24, 25].

The primary mechanism in the defense against oxidative damage relates to the ability of the molecule to scavenge free radicals. Melatonin and another related indole – indole-3-propionic acid (IPA) – have been found to effectively scavenge the highly toxic hydroxyl radical (·OH) with the rate constant approximately of 10^10 M^{−1}s^{−1} [26, 27].

Additionally, melatonin has been shown to directly or indirectly detoxify the following free radicals or ROS: peroxynitrite anion (ONOO−), the superoxide anion radical (O2−), nitric oxide (NO), hydrogen peroxide (H2O2), singlet oxygen (1O2), guanosine radical (G·) [10, 12, 13, 28].

The antioxidant enzymes can antagonize the process of carcinogenesis at its different stages. Melatonin is known to stimulate the activities of several antioxidant enzymes, like superoxide diamutase, γ-glutamylcysteine synthetase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, and catalase (CAT); melatonin also inhibits the activity of a pro-oxidative enzyme, i.e., nitric oxide synthase (NOS) [10, 12, 13, 28].

Ascorbic acid (vitamin C) is a commonly used antioxidant. It is known to provide some protection against tumors [29]. However, ascorbic acid may itself increase oxidative damage, especially in case of co-treatment with transition metal [30].

2. Oxidative damage related to potential carcinogens and protective effects of melatonin and other antioxidants – experimental evidence

2.1. Iron

Iron, as ferrous ion (Fe2+) participates in Fenton reaction – the most basic reaction of oxidative stress (Fe2+ + H2O2 → Fe3+ + ·OH + ·OH). Fenton reaction, during which the most harmful free radical – ·OH – is produced, may accompany all the steps of carcinogenesis [31, 32]. It is known that increased iron stores in the organism are associated with increased risks of cancer [33].

An experimental model of oxidative damage to membranes has been developed, using two substrates for Fenton reaction: Fe2+ and H2O2. In this model, two
products of lipid peroxidation – malondialdehyde + 4-hydroxyalkenals (MDA+4-HDA) – are measured. In an in vitro study, Fe$^{2+} + \text{H}_2\text{O}_2$-related lipid peroxidation was prevented in homogenates of hamster testes in a concentration-dependent manner, by related indoles, i.e., by melatonin, and N-acetylserylserotonin (NAS), the immediate precursor of melatonin, and by 5-hydroxy-indole-3-acetic acid (5HIAA), the main metabolite of serotonin [34]. In another study, we found that melatonin, vitamin E, vitamin C, glutathione and desferrioxamine inhibited Fe$^{2+} + \text{H}_2\text{O}_2$-related lipid peroxidation in liver homogenates in a concentration-dependent manner; additionally, melatonin revealed synergistic effects with other antioxidants [35]. Thus, a supplementation with melatonin and other indoles may prevent iron-induced lipid peroxidation and iron-related sperm abnormalities, both factors contributing to carcinogenesis [7, 8, 36].

It has recently been shown that in vivo treatment with melatonin protects against in vitro iron-induced lipid peroxidation in liver homogenates; in turn, when ascorbic acid was used in that model, no protective effect were observed [37]. Thus, an administration of melatonin to organisms decreases organ susceptibility to oxidative stress after the tissues are oxidatively challenged in vitro.

In another experimental model, FeCl$_3$, ADP and NADPH were used to induce oxidative damage to membranes. Ferric ion (Fe$^{3+}$) indirectly participates in Fenton reaction, when reduced to Fe$^{2+}$. The incubation of microsomal membranes in the presence of FeCl$_3$, ADP and NADPH caused a decrease in membrane fluidity (the inverse of membrane rigidity), accompanied by an increase in amount of lipid peroxidation products; a preincubation with melatonin [38,39], NAS [40], or with other related molecules, like 6-methoxy-1,2,3,4-tetrahydro-β-carboline (pinoline) [41], 5-methoxytryptophol [42], and IPA [43] protected against those oxidative changes. It is worth mentioning that melatonin enhanced the protective effect of tamoxifen, an antiestrogenic drug, used in the treatment of breast cancer, against Fe$^{3+}$-induced membrane oxidative damage [39].

2.2. δ-Aminolevulinic acid

δ-Aminolevulinic acid (ALA) is a precursor of haem synthesis and its increased concentration in blood is related to inherited or acquired porphyrias – acute intermittent porphyria (AIP), hereditary tyrosinemia and lead poisoning [44]. An increased incidence of cancer, especially in liver is observed, in patients suffering from porphyrias [45]. The accumulation of porphyrins or their precursors, followed by free radical generation and the release of iron from its storage sites, are assumed to be responsible for the higher incidence of cancer in porphyric patients.

ALA is used in an experimental model of porphyrin-related oxidative damage and carcinogenesis. In several studies, ALA has been shown to change different parameters of oxidative damage to macromolecules, while melatonin has been found to prevent those changes. Melatonin, when injected to rats, protected against the formation of 8oxodGuo in the liver, kidney, lung and spleen, resulting from a chronic treatment with ALA [46–48]. Similarly, melatonin in vivo prevented the decrease in membrane fluidity in hepatic and renal microsomes and mitochondria [46, 47] and the formation of lipid peroxidation products in hepatic microsomal membranes [46], in lung and spleen homogenates [48] and in blood serum [47], due to ALA action.

Several other studies on the protective effects of melatonin against ALA-induced oxidative damage have been published and they have recently been reviewed [28].

2.3. Phenylhydrazine (PHZ)

Phenylhydrazine (PHZ), belonging to the hydrazine family, is one of the most potent toxin used in experimental models of carcinogenesis [49]. PHZ intoxication leads, among others, to hepatic and spleen iron overload, free iron release, followed by free radical generation [50]. A chronic treatment with PHZ resulted in a pronounced increase in lipid peroxidation products in spleen and serum; those changes were prevented by melatonin but not by ascorbic acid [37]. Additionally, the pronounced decrease in hepatic membrane fluidity was reduced by melatonin, whereas a co-treatment with ascorbic acid even enhanced the damaging effect of PHZ, resulting in a further decrease in membrane fluidity [37].

2.4. Ionizing radiation (IR)

Radiation injury to living cells is, to a large extent, due to free radical generation [51]. Several studies have been performed, revealing protective effects of melatonin against oxidative abuse, due to ionizing radiation. Total body irradiation (800 Gy) of rats to IR resulted in an increased formation of 8oxodGuo and in a decreased membrane fluidity in liver; a co-treatment with melatonin completely prevented those oxidative changes [52]. Preincubation with melatonin reduced the phenomenon of cell death and decreased lipid peroxidation in cultured human skin fibroblasts, due to exposure to IR [53]. The results of other studies on melatonin and its protective effects against IR-based oxidative damage, have recently been reviewed [13].

2.5. Chromium (Cr)

Chromium (Cr) is used in occupational settings like, e.g., the production of chromates, chromium plating, chromate pigment manufacture, and in the production of cement and stainless steel. The primary toxic form, to which organisms are exposed, is hexavalent Cr (Cr$^{6+}$) [54]. The carcinogenic activity of Cr is thought to be the result of macromolecular damage caused, by reactive intermediates, arising in the course of its intracellular reduction of Cr$^{6+}$ to trivalent Cr (Cr$^{3+}$) and/or by Cr$^{3+}$ itself; Cr$^{3+}$ does not cross cellular membranes and accumulates in cells [54].

An incubation of primary cultures of rat hepatocytes in the presence of Cr$^{6+}$, caused DNA single-strand breaks, cellular toxicity, measured by the leakage of lactate dehydrogenase from cells, and an increased level of lipid peroxidation products [55]. Melatonin prevented Cr$^{6+}$-related oxidative changes and restored the levels of anti-
oxidants – vitamins C and E, and the activity of CAT [55]. In another study, Cr³⁺ plus H₂O₂ induced an increase in 8oxodGuo formation in purified calf thymus DNA and that effect was prevented by melatonin, applied in micromolar concentrations [56, 57]; other related molecules – IPA, pinoline, NAS and 6-hydroxymelatonin – provided a somewhat lower protection [56], whereas ascorbic acid and trolox (a water soluble form of vitamin E) were about 60–70 times less effective [57] than melatonin in reducing DNA oxidative damage in that in vitro model. In a similar experimental model, the decrease in membrane fluidity of rat hepatic microsomal membrane, caused by Cr³⁺+H₂O₂, was prevented by IPA [58].

2.6. Cadmium (Cd)

Cadmium (Cd), a toxic transition metal, is widely used in occupational settings, such as smelting, refining of zinc, electroplating, galvanizing, nickel-cadmium battery production, welding, and it is also present in tobacco. Cd-related depletion of glutathione and the induction of lipid peroxidation are thought to play a substantial role in Cd-related carcinogenesis [59]. Cadmium, when applied in single injection, enhanced lipid peroxidation in different hamster organs – brain, heart, kidney and lung; those changes were prevented by a co-treatment with melatonin [60]. Thus, this indoleamine may be regarded as a potential anticarcinogenic factor which, while reducing Cd-related lipid peroxidation, may effectively protect against any further, more harmful damages, resulting from the action of the metal on live organisms.

2.7. Bromine

Potassium bromate (KBrO₃), which is used as a food additive, appeared to be a renal carcinogen in animal models [61]. Its injection to rats resulted in increased levels of 8oxodGuo in kidney; those increases were reduced after a pretreatment with melatonin, as well as by resveratrol and vitamin E [62].

2.8. Mercury

Mercury – a heavy metal – is not only carcinogenic but probably is also involved in the pathophysiological mechanisms of Alzheimer’s disease. Its cytotoxic effects in SH-SY5Y neuroblastoma cells were accompanied by a reduction in cellular glutathione; a pretreatment with melatonin protected cells from mercury-induced GSH loss [63].

2.9. Ferric nitritotriacetate (Fe-NTA)

Nitritotriacetic acid is widely used as a substitute in detergents for household and hospital use, manifesting low toxicity in experimental animals; however, the ferric chelate – ferric nitritotriacetate (Fe-NTA), has been reported to cause a high incidence of renal adenocarcinoma in animal models [64]. Fe-NTA, applied in vitro, increased lipid peroxidation in rat kidney homogenates; similarly, Fe-NTA injected to animals, increased the levels of MDA+4-HDA and of 8oxodGuo [65]. As expected, melatonin prevented the damaging effects of Fe-NTA, both in vitro and in vivo [65].

2.10. Safrole

Safrole is a constituent of several essential oils and is used in perfumery, denaturing fats in soap manufacture, and in the manufacture of heliotropin. Safrole is a complete hepatocarcinogen for rats and mice [66]. When used in animal models, that toxin caused DNA damage. Safrole, applied in vivo, increased DNA adduct formation in rat liver; melatonin, in both pharmacological and physiological concentrations, protected – in dose dependent manner – against safrole-caused DNA damage [67, 68]. Safrole, injected at night, when the blood concentration of melatonin is physiologically higher, caused weaker DNA damage than when injected during the day; conversely, pinealectomy, which eliminates the night-time rise in melatonin concentration, enhanced the formation of DNA adducts [68]. The blood concentration of melatonin was inversely related to the degree of DNA adduct formation induced by safrole [67, 68]. Thus, melatonin, in physiological concentrations, prevented the oxidative damage of carcinogen used in pharmacological concentrations.

2.11. 2-Nitropropane (2-NP)

2-nitropropane (2-NP), the secondary nitroalkane, is widely used as an intermediate in chemical syntheses, in formulation of inks, paints, varnishes, adhesives and other coatings. It is a potent hepatocarcinogenic agent in rodents [69]. Additionally, leukemia and non-Hodgkin’s lymphoma have been described among farmers exposed to solvents including 2-NP [70]. Melatonin significantly reduced the level of lipid peroxidation in rat liver, lung, and kidney and decreased the activity of sorbitol dehydrogenase (related to hepatic damage), stimulated by an earlier single intraperitoneal injection of 2-NP [71].

2.12. Phosphine (PH₃)

Phosphine (PH₃), generated by hydrolysis of metal phosphides (AlP₃, Mg₃P₂), is an important dopant in electronic industry. Genotoxic effects of PH₃ have been described in mice [72] and humans [73]. In animal models, PH₃ increased the level of MDA+4-HDA and decreased GSH concentration in brain, lung, and liver and, additionally, increased the level of 8oxodGuo in brain and lung; melatonin and, to a lesser degree, two other antioxidants – vitamin C and β-carotene, prevented those changes [74].

2.13. 17β-estradiol

1,3,5[10]-Estratriene-3,17β-diol (17β-estradiol; E₂), a natural estrogen, is classified as a carcinogen [75, 76]. An animal model has been developed, relying on an induction of renal tumors in Syrian hamsters, due to chronic exposure to estrogens; this animal model shares the features of estrogen-related tumors in human females, making its use appropriate for investigating the mechanisms of estrogen-related carcinogenesis [76]. There is also hamster kidney model of E₂-induced DNA damage. E₂ applied in a single injection, resulted in an increased level of 8oxodGuo in kid-
neous (at 5 hours) and in liver (at 3 hours); those changes were prevented when the animals were co-treated with melatonin [77]. In another study, we found that not only melatonin, but also IPA and ascorbic acid (used in equimolar doses) prevented the enhanced oxidation of guanine bases; at the same time, however, NAS did not reveal any protective effect against DNA damage, due to E2 [78]. The protective effect of IPA against E2-induced DNA damage is likely related to the similarities in the chemical structures and the antioxidative properties of IPA and melatonin [27]. Concerning ascorbic acid, its protective action is likely related to its ability to reduce redox cycling between estrogens and their corresponding quinone metabolites [29], which are believed to be responsible for the observed oxidative DNA damage and, consequently, for carcinogenesis. Indeed, vitamin C is well known to reduce the incidence of kidney tumors, due to chronic treatment with E2 [29]. Thus, melatonin, IPA and ascorbic acid can be considered as pharmacological agents for the use in co-treatment with estrogens.

3. Concluding remarks
Melatonin effectively reduces several parameters of oxidative damage, due to carcinogens. It is known that melatonin – in physiological concentrations – contributes to the antioxidant capacity of human serum [79]. Thus, this indole, produced in the organism, participates in natural defense mechanisms against oxidative and cancer stress. Therefore, under conditions of exposure to carcinogens or in case of cancer, a supplementation with melatonin or other antioxidants should be considered. It is worth stressing, that high effectiveness of melatonin has recently been found in humans under conditions different from cancer [80].

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Melatonin, oxidative damage, and cancer

Neuroendocrinology Letters ISSN 0172–780X Copyright © 2002 Neuroendocrinology Letters

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