Neuroendocrinology Letters ISSN 0172–780X

REVIEW ARTICLE

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

Malgorzata Karbownik[1,2]

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**

Neuroendocrinology Letters 2002; ***23****(suppl 1):39–44 pii:NEL230702R07 Copyright © Neuroendocrinology Letters 2002*

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

ALA	δ-Aminolevulinic acid
CAT	catalase
Cd	cadmium
Cr	chromium
Cr^{6+}	hexavalent chromium
Cr^{3+}	trivalent chromium
E_2	1,3,5[10]-Estratriene-3,17β-diol (17β-estradiol)
Fe^{2+}	ferrous ion
Fe^{3+}	ferric ion
Fe-NTA	ferric nitrilotriacetate
G·	guanosine radical
5HIAA	5-hydroxy-indole-3-acetic acid
H_2O_2	hydrogen peroxide
IPA	indole-3-propionic acid
IR	ionizing radiation
$KBrO_3$	potassium bromate
MDA+4-HDA	malondialdehyde + 4-hydroxyalkenals
NAS	*N*-acetylserotonin
NOS	nitric oxide synthase
NO·	nitric oxide
2-NP	2-nitropropane
$O_2^{-}\cdot$	the superoxide anion radical
$^1O_2$	singlet oxygen
·OH	hydroxyl radical
ONOO	peroxynitrite anion
8oxodGuo	8-oxo-2'-deoxyguanosine
PH_3	phosphine
PHZ	phenylhydrazine
pinoline	6-methoxy-1,2,3,4-tetrahydro-β-carboline
ROS	reactive oxygen species

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 reffered 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 of 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 ($O_2^{-}\cdot$), nitric oxide ($NO\cdot$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), 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 od several antioxidant enzymes, like superoxide dismutase, 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 (Fe^{2+}) participates in Fenton reaction – the most basic reaction of oxidative stress ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + {}^-OH$). 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: Fe^{2+} and H_2O_2. In this model, two

products of lipid peroxidation – malondialdehyde + 4–hydroxyalkenals (MDA+4-HDA) – are measured. In an *in vitro* study, $Fe^{2+}+H_2O_2$-related lipid peroxidation was prevented in homogenates of hamster testes in a concentration-dependent manner, by related indoles, i.e., by melatonin, and *N*-acetylserotonin (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 $Fe^{2+}+H_2O_2$-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 increasead 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 porphyria-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 cGy) 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, Cr^{3+} plus H_2O_2 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 $Cr^{3+}+H_2O_2$, 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 a 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 ($KBrO_3$), 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 SHSY5Y neuroblastoma cells were accompanied by a reduction in cellular glutathione; a preincubation with melatonin protected cells from mercury-induced GSH loss [63].

2.9. Ferric nitrilotriacetate (Fe-NTA)

Nitrilotriacetic 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 nitrilotriacetate (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 (PH_3)

Phosphine (PH_3), generated by hydrolysis of metal phosphides (AlP, Mg_3P_2), is an important dopant in electronic industry. Genotoxic effects of PH_3 have been described in mice [72] and humans [73].

In animal models, PH_3 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; E_2), 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 numerous mechanistic features with 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 E_2-induced DNA damage. E_2, applied in a single injection, resulted in an increased level of 8oxodGuo in kid-

neys (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 E_2, [78]. The protective effect of IPA against E_2-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 E_2 [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 stress and cancer. 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].

REFERENCES

1 Halliwell B, Gutteridge JM. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch Biochem Biophys 1986; **246**:501–14.
2 Sies H. Strategies of antioxidant defense. Eur J Biochem 1993; **215**:213–9.
3 Dreher D, Junod AF. Role of oxygen free radicals in cancer development. Eur J Cancer 1996; **32A**:30–8.
4 Klaunig JE, Xu Y, Isenberg JS, Bachowski S, Kolaja KL, Jiang J, et al. The role of oxidative stress in chemical carcinogenesis. Environ Health Perspect 1998; **106**:289–95.
5 De Zwart LL, Meerman JHN, Commandeur JNM, Vermeulen NPY. Biomarkers of free radical damage applications in experimental animals and in humans. Free Radic Biol Med 1999; **26**:202–26.
6 Floyd RA. The role of 8-hydroxydeoxyguanosine in carcinogenesis. Carcinogenesis 1990; **11**:1447–50.
7 Marnett LJ. Oxyradicals and DNA damage. Carcinogenesis 2000; **21**:361–70.
8 Burcham PC. Genotoxic lipid peroxidation products: their DNA damaging properties and role in formation of endogenous DNA adducts. Mutagenesis 1998; **13**:287–305.
9 Stadtman ER. Protein oxidation and aging. Science 1992; **257**:1220–4.
10 Reiter RJ. Oxidative damage in the central nervous system: protection by melatonin. Prog Neurobiol 1998; **56**:359–84.
11 Reiter RJ. Oxidative damage to nuclear DNA: Amelioration by melatonin. Neuroendocrinol Lett 1999; **10**:145–50.
12 Reiter RJ, Tan DX, Qi W, Manchester LC, Karbownik M, Calvo JR. Pharmacology and physiology of melatonin in the reduction of oxidative stress in vivo. Biol Signals Recept 2000; **9**:160–71.
13 Karbownik M, Reiter RJ. Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. Proc Soc Exp Biol Med 2000; **225**:9–22.
14 Reiter RJ, Tan DX, Karbownik M. Cholelithiasis, oxidative stress and melatonin. J Pineal Res 2001; **30**:127–128.
15 Terron MP, Ma Marchena J, Shadi F, Harvey S, Lea RW, Rodriguez AB. Melatonin: an antioxidant at physiological concentrations. J Pineal Res 2001; **31**:95–96.
16 Acuña-Castroviejo D, Martin M, Macias M, Escames G, Leon J, Khaldy H, et al. Melatonin, mitochondria and cellular bioenergetics. J Pineal Res 2001; **30**:65–74.
17 Karasek M, Pawlikowski M. Pineal gland, melatonin and cancer. Neuroendocrinol Lett 1999; **20**:139–144.
18 Pawlikowski M, Winczyk K, Karasek M. Oncostatic action of melatonin: facts and question marks. Neuroendocrinol Lett 2002; **23**(suppl 1):24–29.
19 Bartsch C, Bartsch H, Karasek M. Melatonin in clinical oncology. Neuroendocrinol Lett 2002; **23**(suppl 1):36–44.
20 Lissoni P, Rovelli F, Frassineti A, Fumagalli L, Malysheva O, Conti A, Maestroni G. Oncostatic activity of pineal neuroendocrine treatment with the pineal indoles melatonin and 5-methoxytryptamine in untreatable metastatic cancer patients progressing on melatonin alone. Neuroendocrinol Lett 2000; **21**:319–323.
21 Sauer LA, Dauchy RT, Blask DE. Polyunsaturated fatty acids, melatonin, and cancer prevention. Biochem Pharmacol 2001; **61**:1455–1462.
22 Lissoni P, Rovelli F, Malugani F, Bucovec R, Conti A, Maestroni GJ. Anti-angiogenic activity of melatonin in advanced cancer patients. Neuroendocrinol Lett 2001; **22**:45–47.
23 Menendez-Pelaez A, Reiter RJ. Distribution of melatonin in mammalian tissues: the relative importance of cytosolic versus nuclear localization. J Pineal Res 1993; **15**:59–69.
24 Conti A, Conconi S, Hertens E, Skwarlo-Sonta K, Markowska M, Maestroni GJM. Evidence for melatonin synthesis in mouse and human bone marrow cells. J Pineal Res 2000; **28**:193–202.
25 Stefulj J, Hortner M, Ghosh M, Schauenstein K, Rinner I, Wolfler A, Semmler J, Liebmann PM. Gene expression of the key enzymes of melatonin synthesis in extrapineal tissues of the rat. J Pineal Res 2001; **30**:243–247.
26 Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocr J 1993; **1**:57–60.
27 Chyan J, Poeggeler B, Omar RA, Chain DG, Frangione B, Ghiso J, et al. Potent neuroprotective properties against the Alzheimer β-amyloid by an endogenous melatonin-related indole structure, indole-3-propionic acid. J Biol Chem 1999; **274**: 21937–21942.
28 Karbownik M, Reiter RJ. Melatonin protects against oxidative stress caused by δ-aminolevulinic acid: implication for cancer reduction, Cancer Invest 2002; **20**:in press.
29 Liehr JG. Vitamin C reduces the incidence and severity of renal tumors induced by estradiol or diethylstilbestrol. Am J Clin Nutr 1991; **54**:1256S–60S.
30 Rehman A, Collis CS, Yang M, Kelly M, Diplock AT, Halliwell B, et al. The effects of iron and vitamin C co-supplementation on oxidative damage to DNA in healthy volunteers. Biochem Biophys Res Commun 1998; **246**:293–8.
31 Cadet J, Delatour T, Douki T, Gasparutto D, Pouget JP, Ravanat JL, et al. Hydroxyl radicals and DNA base damage. Mutat Res 1999; **424**:9–21.
32 Aust AE, Eveleigh JF. Mechanism of DNA oxidation. Proc Soc Exp Biol Med 1999; **222**:246–52.
33 Stevens RG, Jones DY, Micozzi MS, Taylor PR. Body iron stores and the risk of cancer. N Engl J Med 1988; **319**:1047–52.
34 Karbownik M, Gitto E, Lewinski A, Reiter RJ. Relative efficacies of indole antioxidants in reducing autoxidation and iron-induced lipid peroxidation in testes: implications for cancer initiation. J Cell Biochem 2001; **81**:693–9.
35 Gitto E, Tan DX, Reiter RJ, Karbownik M, Manchester LC, Cuzzocrea S, et al. Individual and synergistic antioxidative actions of melatonin: studies with vitamin E, vitamin C, glutathione and desferrioxamine (desferoxamine) in rat liver homogenates. J Pharm Pharmacol 2001; **53**:1393–401.
36 Möller H, Skakkebaek NE. Risk of testicular cancer in subfertile men: case-control study. Brit Med J 1999; **318**:559–62.
37 Karbownik M, Reiter RJ, Garcia JJ, Tan DX. Melatonin reduces phenylhydrazine-induced oxidative damage to cellular membranes: evidence for the involvement of iron. Int J

Biochem Cell Biol 2000; **32**:1045–54.
38 Garcia JJ, Reiter RJ, Guerrero JM, Escames G, Yu BP, Oh CS, et al. Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. FEBS Lett 1997; **408**:297–300.
39 Garcia JJ, Reiter RJ, Ortiz GG, Oh CS, Tang L, Yu BP, et al. Melatonin enhances tamoxifen's ability to prevent the reduction in microsomal membrane fluidity induced by lipid peroxidation. J Membrane Biol 1998; **162**:59–65.
40 Garcia JJ, Reiter RJ, Karbownik M, Calvo JR, Ortiz GG, Tan DX, et al. N-acetylserotonin suppresses hepatic microsomal membrane rigidity associated with lipid peroxidation. Eur J Pharmacol 2001; **428**:169–75.
41 Garcia JJ, Reiter RJ, Pie J, Ortiz GG, Cabrera J, Sáinz RM, et al. Role of pinoline and melatonin in stabilizing hepatic microsomal membranes against oxidative stress. J Bioenerg Biomembr 1999; **31**:609–16.
42 Garcia JJ, Reiter RJ, Cabrera J, Pie J, Mayo JC, Sáinz RM, et al. 5-Methoxytryptophol preserves hepatic microsomal membrane fluidity during oxidative stress. J Cell Biochem 2000; **76**:651–7.
43 Karbownik M, Reiter RJ, Garcia JJ, Cabrera J, Burkhardt S, Osuna C, et al. Indole-3-propionic acid, a melatonin-related molecule, protects hepatic microsomal membranes from iron-induced oxidative damage: relevance to cancer reduction. J Cell Biochem 2001; **81**:507–13.
44 Kappas A, Sassa S, Anderson KE. The porphyries. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS, editors. The Metabolic Basis of Inherited Diseases. New York: McGraw-Hill; 1983. p. 1300–84.
45 Linet MS, Gridley G, Nyrén O, Mellemkjaer L, Olsen JH, S Keehn, et al. Primary liver cancer, other malignancies, and mortality risks following porphyria: a cohort study in Denmark and Sweden. Am J Epidemiol 1999; **149**:1010–5.
46 Karbownik M, Reiter RJ, Garcia JJ, Tan DX, Qi W, Manchester LC. Melatonin reduces rat hepatic macromolecular damage due to oxidative stress caused by δ-aminolevulinic acid. Biochim Biophys Acta 2000; **1523**: 140–6.
47 Karbownik M, Tan DX, Manchester LC, Reiter RJ. Renal toxicity of the carcinogen δ-aminolevulinic acid: Antioxidant effects of melatonin. Cancer Lett 2000; **161**:1–7.
48 Karbownik M, Tan DX, Reiter RJ. Melatonin reduces the oxidation of nuclear DNA and membrane lipids induced by the carcinogen δ-aminolevulinic acid. Int J Cancer 2000; **88**:7–11.
49 Parodi S, De Flora S, Cavanna M, Pino A, Robbiano L, Bennicelli C, et al. DNA-damaging activity in vivo and bacterial mutagenicity of sixteen hydrazine derivatives as related quantitatively to their carcinogenicity. Cancer Res 1981; **41**:1469–82.
50 Ferrali M, Signorini C, Sugherini L, Pompella A, Lodovici M, Caciotti B, et al. Release of free, redox-active iron in the liver and DNA oxidative damage following phenylhydrazine intoxication. Biochem Pharmacol 1997; **53**:1743–51.
51 Wallace SS. Enzymatic processing of radiation-induced free radical damage in DNA. Radiat Res 1998; **150**:S60–S79.
52 Karbownik M, Reiter RJ, Qi W, Garcia JJ, Tan DX, Manchester LC, et al. Protective effects of melatonin against oxidation of guanine bases in DNA and decreased microsomal membrane fluidity in rat liver induced by whole body ionizing radiation. Mol Cell Biochem 2000; **211**:137–44.
53 Kim BC, Shon BS, Ryoo YW, Kim SP, Lee KS. Melatonin reduces X-ray irradiation-induced oxidative damages in cultured human skin fibroblasts. J Dermatol Sci 2001; **26**: 194–200.
54 Snow ET. Metal carcinogenesis: mechanistic implications. Pharmacol Ther 1992; **53**: 31–65.
55 Susa N, Ueno S, Furukawa Y, Ueda J, Sugiyama M. Potent protective effect of melatonin on chromium(VI)-induced DNA single-strand breaks, cytotoxicity, and lipid peroxidation in primary cultures of rat hepatocytes. Toxicol Appl Pharmacol 1997; **144**:377–84.
56 Qi W, Reiter RJ, Tan DX, Manchester LC, Siu AW, Garcia JJ. Increased levels of oxidatively damaged DNA induced by chromium(III) and H_2O_2: protection by melatonin and related molecules. J Pineal Res 2000; **29**:54–61.
57 Qi W, Reiter RJ, Tan DX, Garcia JJ, Manchester LC, Karbownik M, et al. Chromium(III)-induced 8-hydroxydeoxyguanosine in DNA and its reduction by antioxidants: comparative effects of melatonin, ascorbate, and vitamin E. Environ Health Persp 2000; **108**:399–402.
58 Karbownik M, Garcia JJ, Lewinski A, Reiter RJ. Carcinogen-induced, free radical-mediated reduction in microsomal membrane fluidity: reversal by indole-3-propionic acid. J Bioenerg Biomembr 2001; **33**:73–8.
59 Stohs SJ, Bagchi D, Hassoun E, Bagchi M. Oxidative mechanisms in the toxicity of chromium and cadmium ions. J Environ Pathol Oncol 2000; **19**:201–13.
60 Karbownik M, Gitto E, Lewinski A, Reiter RJ. Induction of lipid peroxidation in hamster organs by the carcinogen cadmium: amelioration by melatonin. Cell Biol Toxicol 2001; **17**:33–40.
61 Kurokawa Y, Maekawa A, Takahashi M, Hayashi Y. Toxicity and carcinogenicity of potassium bromate – a new renal carcinogen. Environ Health Perspect 1990; **87**:309–35.
62 Cadenas S, Barja G. Resveratrol, melatonin, vitamin E, and PBN protect against renal oxidative DNA damage induced by the kidney carcinogen $KBrO_3$. Free Radic Biol Med 1999; **26**:1531–7.
63 Olivieri G, Brack C, Muller-Spahn F, Stahelin HB, Herrmann M, Renard P, et al. Mercury induces cell cytotoxicity and oxidative stress and increases beta amyloid secretion and tau phosphorylation in SHSY5Y neuroblastoma cells. J Neurochem 2000; **74**:231–6.
64 Li J, Okada S, Hamazaki S, Ebina Y, Midorikawa O. Subacute nephrotoxicity and induction of renal cell carcinoma in mice treated with ferric nitrilotriacetate. Cancer Res 1987; **47**:1867–9.
65 Qi W, Reiter RJ, Tan DX, Manchester LC, Kim SJ, Garcia JJ. Inhibitory effects of melatonin on ferric nitriloriacetate-induced lipid peroxidation and oxidative DNA damage in the rat kidney. Toxicology 1999; **139**: 81–91.
66 IARC, Safrole, Isosafrole, and Dihydrosafrole. In: Monographs on the evaluation of the carcinogenic risk of chemicals to man. Lyon: IARC Press. 1976. Vol. 10. p. 231–300.
67 Tan DX, Poeggeler B, Reiter RJ, Chen LD, Chen S, Manchester LC, et al. The pineal hormone melatonin inhibits DNA-adducts formation induced by the chemical carcinogen safrole in vivo. Cancer Lett 1993; **70**:65–71.
68 Tan DX, Reiter RJ, Chen LD, Poeggeler B, Manchester LC, Barlow-Walden LR. Both physiological and pharmacological levels of melatonin reduce DNA adduct formation induced by the carcinogen safrole. Carcinogenesis 1994; **15**:215–8.
69 Fiala ES, Czerniak R, Castonguay A, Conaway CC, Rivenson A. Assay of 1-nitropropane, 2-nitropropane, 1-azoxypropane and 2-azoxypropane for carcinogenicity by gavage in Sprague-Dawley rats. Carcinogenesis 1987; **8**:1947–9.
70 Petrelli G, Siepi G, Miligi L, Vineis P. Solvents in pesticides. Scand J Work Environ Health 1993; **19**:63–5.
71 Kim SJ, Reiter RJ, Garay MVR, Qi W, El-Sokkary GH, Tan DX. 2-Nitropropane-induced lipid peroxidation: antitoxic effects of melatonin. Toxicology 1998; **130**:183–90.
72 Barbosa A, Rosinova E, Dempsey J, Bonin AM. Determination of genotoxic and other effects in mice following short term repeated-dose and subchronic inhalation exposure to phosphine. Environ Mol Mutagen 1994; **24**:81–8.
73 Garry VF, Griffith J, Danzl TJ, Nelson RL, Whorton EB, Krueger LA, et al. Human genotoxicity: pesticide applicators and phosphine. Science 1989; **246**:251–5.
74 Hsu C-H, Han B-C, Liu M-Y, Yeh C-Y, Casida JE. Phosphine-induced oxidative damage in rats: attenuation by melatonin. Free Radic Biol Med 2000; **28**:636–42.
75 Liehr JG. Is estradiol a genotoxic mutagenic carcinogen? Endocrine Rev 2000; **21**: 40–54.
76 Roy D, Liehr JG. Estrogen, DNA damage and mutations. Mutat Res 1999; **424**:107–15.
77 Karbownik M, Reiter RJ, Burkhardt S, Gitto E, Tan DX, A. Lewinski, Melatonin attenuates estradiol-induced oxidative damage to DNA: relevance for cancer prevention. Exp Biol Med 2001; **226**:707–712.
78 Karbownik M, Reiter RJ, Cabrera J, Garcia JJ. Comparison of the protective effect of melatonin with other antioxidants in the hamster kidney model of estradiol-induced DNA damage. Mutat Res 2001; **474**:87–92.
79 Benot S, Goberna R, Reiter RJ, Garcia-Maurino S, Osuna C, Guerrero JM. Physiological levels of melatonin contribute to the antioxidant capacity of human serum. J Pineal Res 1999; **27**:59–64.
80 Gitto E, Karbownik M, Reiter RJ, Tan DX, Cuzzocrea S, Chiurazzi P, et al. Effects of melatonin treatment in septic newborns. Pediatric Res., in press.