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Review Article

# Melatonin: reducing the toxicity and increasing the efficacy of drugs

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## Abstract

Melatonin (*N*-acetyl-5-methoxytryptamine) is a molecule with a very wide phylogenetic distribution from plants to man. In vertebrates, melatonin was initially thought to be exclusively of pineal origin; recent studies have shown, however, that melatonin synthesis may occur in a variety of cells and organs. The concentration of melatonin within body fluids and subcellular compartments varies widely, with blood levels of the indole being lower than those at many other sites. Thus, when defining what constitutes a physiological level of melatonin, it must be defined relative to a specific compartment. Melatonin has been shown to have a variety of functions, and research in the last decade has proven the indole to be both a direct free radical scavenger and indirect antioxidant. Because of these actions, and possibly others that remain to be defined, melatonin has been shown to reduce the toxicity and increase the efficacy of a large number of drugs whose side effects are well documented. Herein, we summarize the beneficial effects of melatonin when combined with the following drugs: doxorubicin, cisplatin, epirubicin, cytarabine, bleomycin, gentamicin, ciclosporin, indometacin, acetylsalicylic acid, ranitidine, omeprazole, isoniazid, iron and erythropoietin, phenobarbital, carbamazepine, haloperidol, caposide-50, morphine, cyclophosphamide and L-cysteine. While the majority of these studies were conducted using animals, a number of the investigations also used man. Considering the low toxicity of melatonin and its ability to reduce the side effects and increase the efficacy of these drugs, its use as a combination therapy with these agents seems important and worthy of pursuit.

## Introduction

Besides their beneficial effects, a number of clinically useful drugs inflict collateral damage when they are administered. Thus, while they may be helpful for a specific condition, at the same time they subvert molecular physiology and cellular function to the extent that they eventually compromise the overall well being of the organism. This review is concerned with those drugs whose damaging effects are mediated by free radicals and related reactants. Currently, there is great interest in the possibility of quelling this biological destruction with the use of agents that quench radical species and their toxic metabolites. Agents that are capable of carrying out these functions are referred to as free radical scavengers or antioxidants (Scandalios 1997; Sies 1997; Cadenas & Packer 2002).

One of the major precursors of free radicals is molecular oxygen ($O_2$, dioxygen). While $O_2$ is obviously an absolute requirement for the survival of aerobic organisms where it functions as an electron sink for energy-yielding processes and as an agent for a variety of metabolic transformations which involve oxidation and oxygenation, at the same time a small percentage (up to an estimated 4%) of the inhaled $O_2$ is converted to damaging metabolites (Grisham 1992). The molecular destruction meted out by these reactants is generically identified as oxidative stress (Sies 1986). The concurrent beneficial and destructive actions of $O_2$ are commonly referred to as the oxygen paradox (Hauptmann & Cadenas 1997).

Oxygen toxicity occurs when $O_2$ is reduced to free radicals and other reactive species

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**Table 1** Commonly discussed reactive species, some of which are free radicals and others that are non-radicals.

| Radicals | Non-radicals |
|---|---|
| Reactive oxygen species (ROS) | |
| Superoxide anion ($O_2^{-\bullet}$) | Singlet oxygen ($^1O_2$) |
| Hydroperoxyl ($HO_2^{\bullet}$) | Hydrogen peroxide ($H_2O_2$) |
| Hydroxyl ($^{\bullet}OH$) | Hypochlorous acid (HOCl)[a] |
| Lipid peroxyl ($LOO^{\bullet}$) | Peroxynitrite anion ($ONOO^-$) |
| Lipid alkoxyl ($LO^{\bullet}$) | Lipid peroxides (LOOH) |
| | Ozone ($O_3$) |
| Reactive nitrogen species (RNS) | |
| Nitric oxide ($NO^{\bullet}$) | Nitrous acid ($HNO_2$) |
| Nitrogen dioxide ($NO_2^{\bullet}$) | Nitrosyl cation ($NO^+$) |
| | Nitroxyl anion ($NO^-$) |
| | Peroxynitrite anion ($ONOO^-$) |
| | Peroxynitrous acid (ONOOH) |

This is only a partial listing and includes those that are most germane to the current review. Reactive oxygen (ROS) and reactive nitrogen species (RNS) are collective terms and include both radical and non-radical species that are oxidizing agents. The term reactive is relative and the specific reactivity varies widely under different conditions and with different substrates. [a]Hypochlorous acid (HOCl) is also classified as a reactive chlorine species

(Table 1). Free radicals are defined as molecules that possess one or more unpaired electrons in their valence orbital. Besides the oxygen-centered radicals and reactants, there are also those that are nitrogen-based (e.g., nitric oxide ($NO^{\bullet}$)), and others. The non-radical reactants do not possess an unpaired electron but, nevertheless, their high reactivity permits them to damage other non-radical species (Table 1) (Halliwell & Gutteridge 1999). The relationship of some of these reactants is summarized in Figure 1. Since $O_2$ utilization by aerobes is by necessity continuous, molecular pummelling from oxygen-derived reactants is also incessant.

Free radical scavengers or antioxidants function as biological bodyguards for essential molecules by either neutralizing reactive species before they mutilate a molecule or they repair the damage that has been inflicted (Acworth et al 1997; Wallace 1997). Antioxidants can either function as direct free radical scavengers or by enzymatically metabolizing the reactants to innocuous species. Some of the best-known antioxidant enzymes include the superoxide dismutases (SOD), the glutathione peroxidases (GSH-Px) and catalase (CAT) (Pippenger et al 1998). This review is concerned with the protective effects of a newly discovered direct scavenger and indirect antioxidant, melatonin, against the toxicity of commonly used pharmaceutical agents.

## Melatonin: reducing oxidative stress

Melatonin (*N*-acetyl-5-methoxytryptamine) is a derivative of the amino acid tryptophan with a phylogenetic ubiquitous distribution. It is found in plants (Balzer & Hardeland 1996; Reiter et al 2001b; Van Tassel et al 2001), bacteria (Manchester et al 1995), unicells (Hardeland et al 1995) and invertebrates (Hardeland & Fuhrberg 1996) as well as

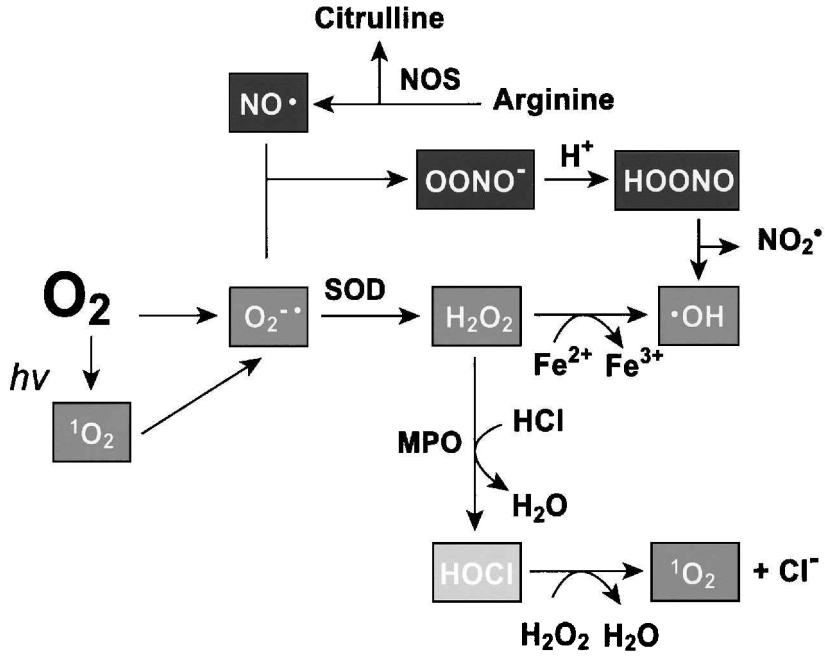


**Figure 1** Free radicals and toxic reactants generated from molecular oxygen ($O_2$). Two to four percent of the $O_2$ inhaled by aerobes is reduced to free radicals such as the superoxide anion ($O_2^{-\bullet}$) and highly toxic hydroxyl radical ($^{\bullet}OH$); an intermediate in this process is the reactant hydrogen peroxide ($H_2O_2$). The photoexcitation of $O_2$ produces singlet oxygen ($^1O_2$) which is also capable of damaging molecules. Besides the $O_2$-based reactants (red), nitrogen-centred molecules (blue) also exhibit significant toxicity. Hypochlorous acid (HOCl, orange) is classified either as an oxygen or chlorine-based reactant.

in vertebrates (Reiter 1991) including man (Brzezinski 1997). In mammals, it is likely produced in a variety of organs and cells (Bubenik 2001; Stefulj et al 2001) but it is best known as the major secretory product of the pineal gland (Reiter 1991), an organ whose physiological roles have only been uncovered in the last several decades. Of significance to the current review is that in all species where it has been measured, melatonin levels wane with increasing age such that, in the elderly, melatonin concentrations in the blood are only a fraction of those in the young (Reiter 1992). Functionally, melatonin has been linked to the regulation of seasonal reproduction (Reiter 1980), strengthening of circadian rhythms (Arendt 1988), stimulation of the immune system (Guerrero & Reiter 1992, 2002; Maestroni 2001), inhibition of cancer initiation (Reiter 1999; Karbownik & Reiter 2000) and tumour growth (Blask et al 1991; Sauer et al 2001) and sleep processes (Garfinkel et al 1995; Dijk & Cajochen 1997). That melatonin functions as a powerful free radical scavenger and antioxidant was only uncovered in the last decade (Poeggeler et al 1993; Tan et al 1993; Hardeland et al 1995; Reiter et al 1995, 2002a). Considering the diminished melatonin production in aged organisms (Reiter 1992), the functions of melatonin are likewise attenuated in the elderly.

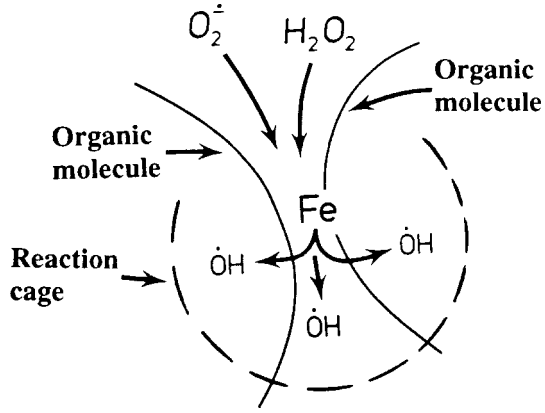


**Figure 2** Hydroxyl radicals (·OH) can be generated in all subcellular compartments and when they are, they have the capability of damaging any neighbouring organic molecule (i.e., protein, lipid, DNA, etc.). Because of its very high reactivity, ·OH migrates a very short distance before mutilating a molecule, damaging it within what has been referred to its reaction cage; this is referred to as on site damage. Unlike some other reactants, ·OH is not enzymatically metabolized to non-reactive substances and can only be neutralized by direct free radical scavengers. For a scavenger to prevent ·OH-mediated damage, it must be within the reaction cage when the radical is generated.

## Melatonin: direct scavenging actions

Hundreds of reports have appeared in the last 8 years that have documented melatonin's ability to directly neutralize free radicals and related toxicants. The bulk of the reactants listed in Table 1 have been shown to be detoxified by melatonin. The first indication that melatonin may be a direct free radical scavenger actually appeared in 1991, but the details relating to what was done and the specific findings are difficult to unravel because of incomplete methodological details and of the poor English composition of the report (Ianas et al 1991). Soon thereafter, however, definitive evidence that melatonin functioned as a direct scavenger of hydroxyl radicals (·OH) was provided (Tan et al 1993; Poeggeler et al 1994). For these studies, $H_2O_2$ was exposed to 254 nm ultraviolet light to generate ·OH that was captured with the spin-trapping agent 5,5-dimethyl-pyrroline-*N*-oxide (DMPO). The resulting adducts (i.e., ·OH-DMPO) were identified and quantified using electron spin resonance (ESR) spectroscopy, widely accepted as the most definitive method for identifying such adducts. When melatonin was added to the mixture in increasing concentrations, it progressively reduced ·OH-DMPO, proving it had scavenged ·OH so it was no longer available for adduct formation. At that time, we also proposed a reaction pathway for melatonin with the ·OH (Hardeland et al 1993).

In 1998 it was shown that each melatonin molecule actually scavenges two ·OH and generates the product cyclic 3-hydroxymelatonin (cyclic 3-OHM) (Tan et al 1998). Cyclic 3-OHM was identified by mass spectrometry/gas chromatography (GCMS) and proton nuclear magnetic resonance ($^1$H NMR). That the reaction of melatonin with ·OH occurs in-vivo was confirmed by the measurement of cyclic 3-OHM in the urine of rodents and man. In these studies, the quantity of urinary cyclic 3-OHM was increased when rats were supplemented with melatonin and rose even further when the rats were exposed to whole-body ionizing radiation, a physical agent known to generate ·OH. Many other studies have confirmed melatonin's ability to detoxify ·OH (Matuszak et al 1997; Susa et al 1997; Bandyopadhyay et al 2000; Brömme et al 2000; Poeggeler et al 2002). The calculated biomolecular rate constant for the melatonin/·OH reaction is $0.6 \times 10^{11}$ M$^{-1}$ s$^{-1}$ (Poeggeler et al 1996).

The significance of melatonin as an ·OH scavenger relates to the fact that this reactant is generally considered to be the most damaging of all endogenously generated reactive agents. Once produced, it plunders any molecule it encounters in its immediate vicinity (Figure 2). Indeed, its high reactivity prevents it from moving more than a few molecular diameters from where it was produced before it biochemically alters a neighbouring molecule. Thus, for any scavenger to combat ·OH-mediated damage it must be essentially at the site where the radical is produced to prevent its destructive actions. Unlike some other well-known antioxidants that are exclusively lipid (e.g., vitamin E) or water (e.g., vitamin C) soluble and, therefore, exhibit a limited intracellular distribution, melatonin is amphiphilic allowing it to reduce ·OH-mediated damage in both the lipid and aqueous subcellular compartments. Evidence has shown that melatonin is clearly highly soluble in a lipid-based medium (Costa et al 1997) and it has also been shown to dissolve in aqueous media (Shida et al 1994). Rather little is known concerning melatonin's specific intracellular distribution or concentration, although the few studies that have been performed suggest that its deposition in different subcellular compartments is not

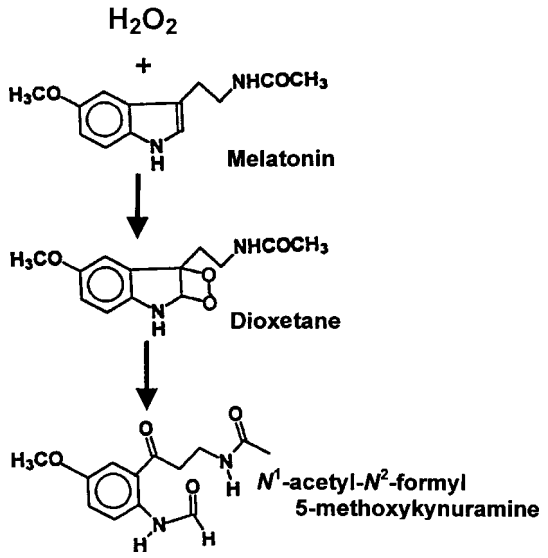


**Figure 3** In a pure chemical system, melatonin has been shown to directly interact with hydrogen peroxide ($H_2O_2$) with the formation of $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine (AFMK). AFMK is also an effective free radical scavenger that thereby increases the efficacy of melatonin as an antioxidant. The sequence whereby melatonin and its resulting products are all scavengers is referred to as the cascade reaction. Dioxetane has been proposed as an intermediate in this reaction.

equal (i.e., its levels in any single compartment are neither in equilibrium with its concentrations in an adjacent organelle nor in equilibrium with melatonin levels in the blood) (Menendez-Pelaez et al 1993; Tan et al 1999a, b; Conti et al 2000; Reiter et al 2001a; Skinner & Malpaux 2000) (also see below).

The $^{\bullet}OH$ is not the only oxygen-derived reactant that is neutralized by melatonin. Recently, it was documented that, in fact, the immediate precursor of $^{\bullet}OH$ (i.e., hydrogen peroxide ($H_2O_2$); Figure 1) is also scavenged by melatonin (Tan et al 2000a). Usually when one considers the removal of $H_2O_2$ from cells, it is via its enzymatic conversion to innocuous agents. The two major enzymes involved in these conversions are the GSH-Px and CAT. Recent evidence, however, has uncovered a pathway whereby melatonin directly interacts with $H_2O_2$ to diminish its levels in a pure chemical system (Tan et al 2000a). The product that results from the melatonin–$H_2O_2$ interaction is $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine (AFMK) (Tan et al 2001) (Figure 3). Additionally, AFMK was shown to be capable of donating two electrons and, therefore, being a direct free radical scavenger in its own right. In specific experiments, AFMK reduced DNA damage induced by a combination of $H_2O_2$ and the transition metal, $Cr^{3+}$, and also limited the destruction of lipids resulting from their exposure to $H_2O_2$ and another transition metal, ferrous ion ($Fe^{2+}$) (Tan et al 2001). Collectively, the findings summarized here illustrate that not only does melatonin detoxify $O_2$ by-products, but also one of the resulting metabolites (i.e., AFMK) likewise neutralizes them. This has been referred to as the antioxidant cascade and allows melatonin and its metabolites to scavenge additional radicals beyond what the parent molecule, in this case melatonin, is capable of doing (Tan et al 2000b). This metabolic cascade permits melatonin to directly or indirectly scavenge a number of radicals unlike the classic antioxidants where the ratio of scavenger to radicals neutralized is 1:1. There is evidence that a hepatic enzymatic degradation product of melatonin (i.e., 6-hydroxymelatonin) (Reiter 1991) is also an effective scavenger (Pierrefiche et al 1993; Matuszak et al 1997). This would further expand melatonin's repertoire as an antioxidant.

Singlet oxygen ($^1O_2$) is formed in cells by the photosensitization reaction of substrates such as dyes and biological pigments (Figure 1). Indirect evidence suggesting that melatonin neutralized $^1O_2$ was first provided by Cagnoli et al (1995) when they showed that the brain of photosensitized rose-bengal-treated rats was protected by melatonin administration. In pure chemical systems, Poeggeler et al (1994) found that riboflavin catalysed the oxidation of melatonin and the autoxidation of melatonin during bright light exposure. Likewise, melatonin reduced $^1O_2$-dependent 2,2,6,6-tetramethyl-piperdine oxide radical generation during rose bengal photodynamic excitation (Zang et al 1998).

The superoxide anion radical ($O_2^{-\bullet}$) is the metabolite generated when $O_2$ is reduced by a single electron (Figure 1). It is known to be formed during the respiratory burst of phagocytic cells and during enzyme-catalysed reactions and in mitochondria when $O_2$ is reduced to water. $O_2^{-\bullet}$ is far less reactive than $^{\bullet}OH$ but it quickly couples with nitric oxide ($NO^{\bullet}$) to form the highly toxic peroxynitrite anion ($ONOO^-$), and it is enzymatically dismutated to $H_2O_2$ and forms $^{\bullet}OH$ in the Haber–Weiss reaction.

Melatonin seems to be minimally reactive with $O_2^{-\bullet}$ (Chan & Tang 1996; Marshall et al 1996) although one study, in which ESR was used to identify DMPO-$O_2^{-\bullet}$ adducts, melatonin was reported to be modestly interactive with $O_2^{-\bullet}$ (Zang et al 1998).

One of the most extensively studied processes in free radical biology is lipid peroxidation wherein the peroxyl radical ($LOO^{\bullet}$) is generated; this radical then oxidizes another adjacent lipid molecule to maintain the chain reaction of lipid peroxidation. Early studies by Pieri et al (1994, 1995) claimed that melatonin was a more efficient $LOO^{\bullet}$ scavenger than is vitamin E, considered the premier chain-breaking antioxidant. This claim, however, has not been verified in subsequent studies (Livrea et al 1997; Antunes et al 1999). Thus, it appears that melatonin's ability to reduce lipid peroxidation in-vivo is probably not related to its function as a chain-breaking antioxidant but could be associated with melatonin's ability to scavenge the initiating radicals (Reiter et al 2000a) and to other actions within the molecular lipid bilayer (Garcia et al 1997, 1998; Tesoriere et al 1999). While melatonin seems not be have any particular ability to scavenge the lipid peroxyl radical, it does neutralize the trichloromethylperoxyl radical, an interaction that has a rate constant of $2.7 \times 10^8$ $M^{-1}$ $s^{-1}$ (Marshall et al 1996).

Hypochlorous acid (HOCl) is classified either as an oxygen- or chlorine-based reactant (Table 1; Figure 1); it

is produced in-vivo by activated neutrophils via the catalytic activity of myloperoxidase (MPO). HOCl is a powerful oxidizing agent that has the capability of damaging a variety of molecules. According to Dellegar et al (1999), melatonin inactivates HOCl and in the process 2-hydroxymelatonin, reportedly, is formed. Since the reaction of melatonin with HOCl is made more efficient in the presence of water, this reaction could be of considerable importance in the cytosol of cells.

While the studies summarized above strongly support melatonin's efficacy as a scavenger of oxygen-based reactants, some of the studies were conducted using pure chemical systems. The oxidation chemistry of melatonin in these systems can vary widely according to how the radical and non-radical reactants were generated (Burkhardt et al 2001). Thus, precisely how the findings in these concoctions, in the absence of cells, relate to melatonin's activity in complex in-vivo systems remains unknown.

In-vivo, roughly 4% of the molecular $O_2$ taken into the organism is normally converted to the $O_2$ by-products summarized above. Thus, the greater the concentration of $O_2$ in the inspired air, the more extensive is the molecular damage to the organism. This damage becomes readily apparent when animals (or humans) are exposed to 100% $O_2$ at increased atmospheric pressure (Jenkinson et al 1989). Given that melatonin neutralizes $O_2$-derived reactants, this indole would be expected to protect against hyperbaric hyperoxia exposure. This has been shown to be the case. In both the lungs and the brain, the two organs that are most sensitive to increased $O_2$ levels, melatonin given to rats exposed to 4 atmospheres of 100% $O_2$ for 90 min reduced the breakdown of polyunsaturated fatty acids and maintained high antioxidant enzyme activity (Pablos et al 1997b). The protection afforded by melatonin against $O_2$ toxicity is consistent with the in-vivo free radical scavenging and antioxidant properties of melatonin.

Oxygen-derived reactants are by no means the only intracellularly generated pariahs (Figure 1). The nitrogen-based peroxynitrite anion ($ONOO^-$) or its metabolites (e.g., peroxynitrous acid (ONOOH); Figure 1) are almost equivalent to $^{\bullet}OH$ in terms of destructive capacity. In tests of melatonin's proficiency to scavenge $ONOO^-$, it met the challenge and neutralized this reactant (or its metabolites) (Gilad et al 1997; Zhang et al 1998, 1999; Blanchard et al 2000). Likewise, in many situations where $ONOO^-$ was induced in-vivo, exogenously administered melatonin curtailed the molecular and physiological damage that normally accompanies $ONOO^-$ exposure (Cuzzocrea & Reiter 2001; Dugo et al 2001).

Against other reactants shown in Figure 1, melatonin is also effective in directly interacting with them thereby limiting their ability to mangle essential macromolecules. Thus, besides the agents summarized above, melatonin also has been shown to incapacitate nitric oxide ($NO^{\bullet}$) (Mahal et al 1999; Noda et al 1999; Blanchard et al 2000; Turjanski et al 2000a, b). Although inherently relatively unreactive, $NO^{\bullet}$ quickly couples with $O_2^{-\bullet}$ to form $ONOO^-$ (Beckman et al 1990) that is capable of meting out significant molecular destruction (Villa et al 1994; Phelps et al 1995). Thus, by scavenging $NO^{\bullet}$, melatonin indirectly limits oxidative stress. Besides directly scavenging $NO^{\bullet}$, melatonin curtails its generation under some circumstances by inhibiting the activity of its rate-limiting enzyme, nitric oxide synthase (NOS) (Pozo et al 1997; Crespo et al 1999).

## Melatonin: indirect antioxidant actions

Whereas the repertoire of melatonin as a direct free radical (and associated reactants) scavenger is obviously extensive, precisely how these reactions relate to the indole's ability to protect against such a wide variety of toxicants in-vivo remains to be resolved. This relates to the fact that melatonin, in addition to its ability to directly neutralize reactive species, also limits their generation or metabolizes intermediates to innocuous products. As noted above, melatonin inhibits NOS (Pozo et al 1997; Crespo et al 1999) under some circumstances which lowers tissue damage that is a consequence of either $NO^{\bullet}$ itself or of the product formed (i.e., $ONOO^-$), when it couples with $O_2^{-\bullet}$. Furthermore, melatonin stimulates several important antioxidative enzymes (activity or gene expression) including SOD (Antolin et al 1996; Kotler et al 1998; Albarran et al 2001), GSH-Px (Barlow-Walden et al 1995; Pablos et al 1995, 1997a; Okatani et al 2001; Wakatsuki et al 2001) and glutathione reductase (GSH-Rd) (Pablos et al 1997a). SOD functions as an antioxidant by rapidly removing $O_2^{-\bullet}$ from cells thereby lowering the formation of the highly reactive and damaging $ONOO^-$ (Beckman et al 1990). $H_2O_2$, the product enzymatically produced when SOD reduces $O_2^{-\bullet}$, is the immediate precursor of $^{\bullet}OH$ (Figure 1), is converted to non-reactive products by two important antioxidative enzymes, GSH-Px and CAT, which are differently distributed within tissues. As with SOD, GSH-Px and GSH-Rd, CAT activity is also stimulated by melatonin (Montilla et al 2000; Reiter et al 2000b).

The enzyme GSH-Px utilizes reduced glutathione (GSH), an intracellular thiol that is typically in millimolar concentrations, as a substrate. Maintaining high intracellular concentrations of GSH seems also to be a function of melatonin since this indole stimulates the activity of its rate-limiting enzyme, gamma-glutamylcysteine synthase (Urata et al 1999). When GSH is metabolized by GSH-Px, a reaction that also requires $H_2O_2$ or other hydroperoxides, it is converted to oxidized glutathione (GSSG). Within cells the GSH : GSSG ratio is normally greatly in favour of the former, and to maintain this ratio GSSG is rapidly metabolized back to GSH by GSH-Rd. As noted above, experimental evidence has shown that melatonin also promotes the activity of GSH-Rd thereby helping to maintain high levels of reduced glutathione (Hara et al 2001).

Finally, as mentioned above, melatonin prevents the reduction in another important $H_2O_2$ metabolizing enzyme, CAT (Reiter et al 2000b; Taskiran et al 2000). Thus, the activity of the two antioxidative enzymes which play a major role in maintaining low steady-state concentrations of $H_2O_2$, thereby reducing $^{\bullet}OH$ generation, is normally stimulated by melatonin, as is the recycling of the essential intracellular thiol antioxidant GSH.

The mechanisms whereby melatonin stimulates the activity of the enzymes that detoxify oxygen-based reactants

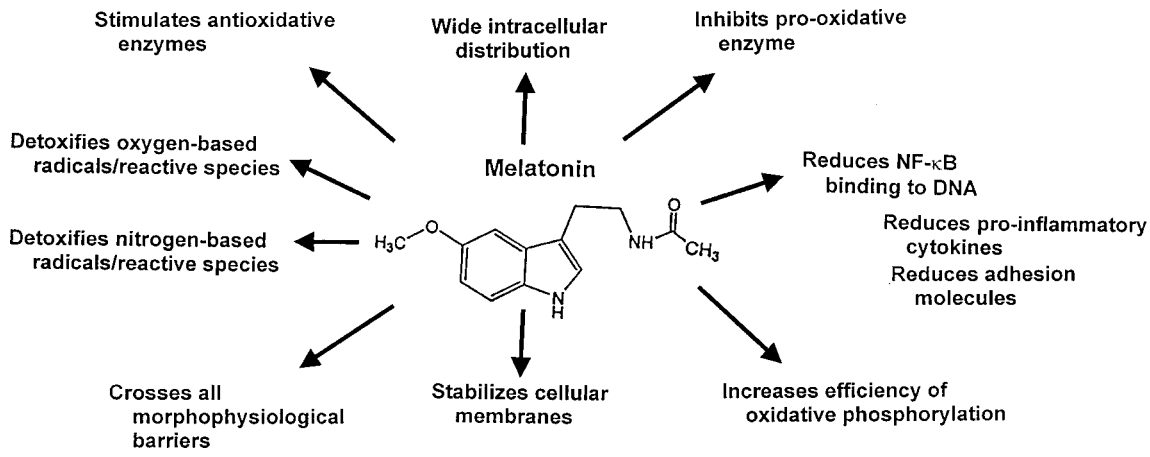


**Figure 4** Melatonin has multiple actions in protecting against drug toxicity. Besides its ability to directly detoxify free radicals and related reactants, melatonin has a variety of other actions that enhance its capacity to reduce molecular damage caused during the metabolism of drugs.

remain unknown but it is likely to be mediated via specific receptors. Although membrane receptors for melatonin have been identified in many cells (Reppert 1997; Dubocovich et al 1999), nuclear binding sites for the indole have also been documented (Garcia-Mauriño et al 1998; Guerrero et al 2000). Either, or both, of these may be involved in the mechanisms by which melatonin promotes the activity of SOD, GPx and GRd. Indeed, in one case, Pablos et al (1997a) showed that a nuclear melatonin receptor agonist, like melatonin itself, stimulated both GPx and GRd in mouse brain. Whether this is a common mechanism by which melatonin increases the activity of these enzymes (or prevents their decrease in high oxidative stress conditions) remains to be investigated. In reference to the stimulation of GSH synthesis as reported by Urata et al (1999), it is assumed that this action of melatonin likewise involves a receptor-mediated process although the location of the receptor remains unresolved.

Beyond these actions, melatonin has additional means whereby it curtails the oxidative mutilation of essential macromolecules. Melatonin maintains the optimal fluidity of cellular membranes (Garcia et al 1997, 1998). This is accomplished by reducing the peroxidation of inherent polyunsaturated fatty acids (PUFA) and indirectly reducing increased membrane rigidity (Garcia et al 1998) by positioning itself within cellular membranes to restrict damage to PUFA by toxic reactants (Tesoriere et al 1999).

Even more important in reducing oxidative damage may be melatonin's action at the level of the electron transport chain (ETC) in mitochondria (Acuña-Castroviejo et al 2001). In addition to the indirect evidence suggesting an action of melatonin on mitochondrial electron transfer (Yamamoto & Tang 1996a, b; Gilad et al 1997), several studies have shown that the indole directly stimulates mitochondrial enzyme activity associated with oxidative phosphorylation (OXPHOS); these include NADH-coenzyme Q reductase (Complex I) and cytochrome c oxidase (Complex IV) (Absi et al 2000; Martin et al 2000a, 2000b). Additionally, besides stimulating OXPHOS that could reduce election leakage and free radical generation (Acuña-Castroviejo et al 2001), melatonin treatment of rat brain and liver mitochondria in-vitro increased ATP production (Martin et al 2002). This action of melatonin may be highly significant in reducing accumulated oxidative damage by providing energy for molecular repair processes. How melatonin influences the mitochondrial enzymes and processes remains enigmatic.

Clearly, the number of mechanisms at melatonin's disposal to reduce molecular destruction and cellular dysfunction due to oxygen- and nitrogen-based reactants is extensive. These actions have been shown, for the most part, to be operative in both in-vitro and in-vivo situations and are summarized in Figure 4. What remains unknown is the relative importance of each of these actions in reducing the toxicity of drugs and toxins to which animals and humans are normally exposed.

Finally, the importance of a number of endogenously generated melatonin metabolites in terms of their scavenging actions should not be overlooked since they likely contribute via the antioxidant cascade defined above to the total capacity of melatonin to reduce oxidative damage. Since this brief summary of melatonin's numerous antioxidative actions does not do the subject justice, the reader may consult more extensive reviews for greater details on melatonin's mechanism and actions in protecting against oxidative stress resulting from drug exposure (Hardeland et al 1995; Reiter et al 2000a, 2001c; Tan et al 2000b, 2002; Cuzzocrea & Reiter, 2001; Livrea et al 2002).

## Melatonin: detoxifying drugs

### *Doxorubicin*

Doxorubicin (adriamycin) is a member of the anthracycline class of cytostatic antibiotics that has been successfully used to treat a variety of soft and solid tumours. Its use, however, is complicated by significant acute and chronic side effects (Sivelski-Iliskovic et al 1994). One of the major long-term consequences of doxorubicin is the development of cardiomyopathy and ultimately congestive heart failure

(Singal & Iliskovic 1998). The molecular mechanisms which account for doxorubicin-induced cardiac damage have been at least partially identified and the generation of $O_2$-based reactants are considered a significant aspect of the resulting cardiopathology (Kaul et al 1993; Gille & Nohl 1997). This information was used as the rationale for testing the efficacy of melatonin in reducing doxorubicin-mediated tissue destruction and malfunction.

The first workers to test the efficacy of melatonin in lowering doxorubicin toxicity in the heart were Morishima et al (1998). They performed detailed chemical and physiological analyses of the heart of rats treated with doxorubicin alone or in combination with melatonin; they also compared the relative protective effects of melatonin with probucol, a cholesterol-lowering drug that has reported beneficial effects in doxorubicin-treated rats. Melatonin and probucol were equally effective in maintaining heart weight, reducing mortality, lowering the volume of ascites fluid, increasing the systolic and diastolic arterial pressures and reversing ultrastructural damage to the cardiomyocytes caused by doxorubicin. While both melatonin and probucol lowered the accumulation of lipid peroxidation products in cardiac tissue, melatonin was more effective in restraining plasma levels of damaged lipid products.

In a follow-up study, the same group (Morishima et al 1999) reported that melatonin's beneficial actions against doxorubicin cardiotoxicity may relate to its ability to modulate zinc distribution; zinc may itself function as an antioxidant. Following doxorubicin treatment, myocardial zinc concentrations typically increase while plasma zinc levels drop. Melatonin prevented the accumulation of zinc in the heart and the drop in plasma levels induced by doxorubicin. Probucol was less effective than melatonin in redistributing zinc during doxorubicin toxicity. Based on the results of their two reports, Morishima et al (1998, 1999) feel melatonin is a better protector of the heart against doxorubicin toxicity than is probucol. In both these studies, the dose of probucol was slightly higher than that of melatonin but for neither drug was a dose–response study performed.

Melatonin has also been compared with a classical antioxidant, vitamin E, relative to its ability to reduce doxorubicin cardiomyopathy (Abdel-Wahab et al 2000). While both melatonin and vitamin E were found to protect, equally effectively, the heart against the toxic reactions of doxorubicin using a number of parameters indicative of oxidative stress, a direct comparison was not valid since the dose of vitamin E used in the studies was 50 times that of melatonin. Also, in this investigation the authors (Abdel-Wahab et al 2000) considered how well melatonin and vitamin E functioned as antitumour agents in combination with doxorubicin. In this test melatonin, again at a 50-fold lower dose than vitamin E, improved the antitumour activity more than did the vitamin antioxidant.

Additional studies relating to the ability of melatonin to protect the heart from doxorubicin toxicity have come from the laboratories of Xu et al (2001a, b) and Agapito et al (2001). Consistent with the observations discussed above, in both in-vivo and in-vitro experiments, Xu et al (2001a, b) showed that pharmacological concentrations of melatonin protected the heart and cardiomyocytes, respectively, from toxic doses of doxorubicin. In particular, melatonin reduced doxorubicin-induced lipid peroxidation, $\beta$-adrenergic receptor alterations and enzyme (lactate dehydrogenase) leakage in cardiomyocytes. Furthermore, melatonin improved cardiac function that was impaired following doxorubicin treatment (Xu et al 2001b). Similarly, Agapito et al (2001) observed that the cardiac GSH/GSSG ratio, which was reduced by doxorubicin, was re-established by melatonin co-treatment and likewise the depressed GSH-Px activity was restored by the indole. Thus, in each of the reports in which melatonin was tested as a drug to reduce doxorubicin toxicity at the level of the heart, regardless of the endpoint assessed, the study was successful in documenting the protection.

The heart is by no means the only organ that suffers from the adverse effects of doxorubicin. The kidney, brain, liver, lung and intestine, as well as the bone marrow, are additional tissues that are morphologically and physiologically damaged as a consequence of free radicals and related metabolites generated following doxorubicin administration. In the kidney, doxorubicin was found to lower GSH levels and to increase lipid hydroperoxides and to cause a marked proteinuria, with each of these negative changes being markedly reduced by melatonin (Montilla et al 1997a, 1998). In addition to maintaining normal levels of GSH, melatonin prevented the accumulation of GSSG and the drop in renal GSH-Px normally accompanying doxorubicin metabolism (Agapito et al 2001). In the rat brain, an organ rich in easily oxidizable lipids, melatonin prevented the accumulation of lipoperoxides following doxorubicin administration (Montilla et al 1997b). In the liver of rats, melatonin reversed the drop in GSH caused by doxorubicin but did not totally reverse the elevated levels of lipid breakdown products (Rapozzi et al 1999). Melatonin did, however, reduce bone-marrow and lymphocyte toxicity (Rapozzi et al 1998) that accompanied administration of the anthracycline drug (Rapozzi et al 1999).

Importantly, as noted above in the report of Abdel-Wahab and colleagues (2000), Granzotto et al (2001) noted that melatonin does not interfere with the action of doxorubicin on tumour cells but, rather, actually enhances the effect of the anticancer drug possibly by inhibiting P-glycoprotein-mediated doxorubicin efflux from cancer cells. This is consistent with our own proposal that melatonin, because of its combined actions as an antioxidant and tumour suppressor, may exaggerate the anticancer efficacy of doxorubicin (Figure 5) (Reiter et al 2002b).

*Cisplatin*

Cisplatin (*cis*-diamminedichloroplatinum), like doxorubicin, is a widely used chemotherapeutic agent, the dose and use of which is limited by its toxicity. The tissues most readily damaged by cisplatin include the kidneys (Kociba & Sleight 1971), peripheral nerves (Ozols et al 1983) and structures of the inner ear (Stadnicki et al 1975). The biochemical basis of cisplatin toxicity is believed to relate to free radicals generated in these tissues (Ravi et al 1995). Indeed, several antioxidants have been tested, with limited

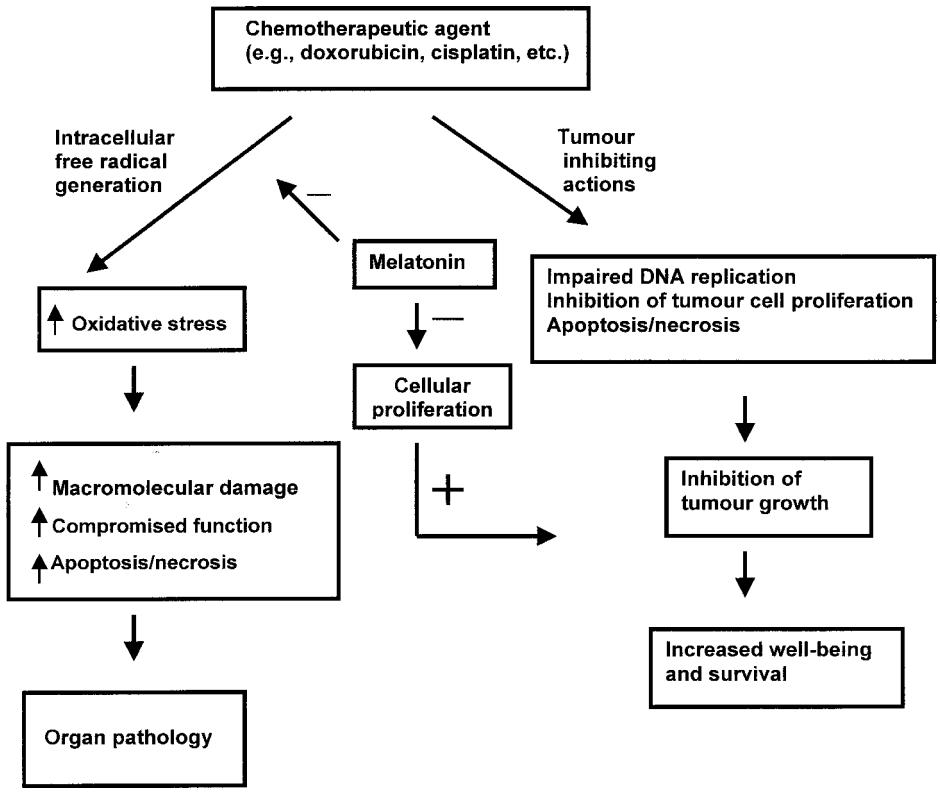


**Figure 5** Doxorubicin and a number of other chemotherapeutic agents inhibit tumour growth by mechanisms that are drug specific. Additionally, however, they generate free radicals that cause extensive molecular damage or dysfunction that can lead to organ pathology and, in the worst case scenario, to organismal death. By functioning as a ubiquitously acting antioxidant melatonin can reduce (−) the free-radical-mediated damage while also enhancing (+) tumour inhibition due to its own intrinsic tumour-inhibiting activity; tumour inhibition by melatonin may involve reducing cellular proliferation as well as other processes.

success, as potential agents to ameliorate the destructive actions of this chemotherapeutic drug (Rybak et al 1995). Recent studies have also used melatonin as a potential mitigating agent against the collateral damage caused by cisplatin.

Following their demonstration that melatonin is normally found in tissues (membraneous cochlea and modiolus) of the inner ear, Lopez-Gonzalez et al (1997) examined the efficacy of exogenously administered melatonin on the ototoxicity of cisplatin in rats (Lopez-Gonzalez et al 2000b). In this case melatonin was either subcutaneously injected or administered in the drinking water with the end-point being the measurement of distortion product otoacoustic emissions (DPOAEs) measured via a probe placed in the rats' external auditory canal; the record of the distortion product is known as a DPgram. Cisplatin administration to rats caused functional damage to the auditory apparatus as evidenced by the recorded Dpgrams, with melatonin, given by either route, being protective at all frequencies tested. In this study the authors used, in addition to melatonin, an antioxidant mixture, the combined dose of which was much greater than that of melatonin. Despite this, the mixture was no more effective in reducing cisplatin toxicity than was the indole given alone at a much lower dose. The beneficial effects of melatonin at the level of the inner ear were also shown by the ability of melatonin to cause a 3.5-fold prolongation of otoacoustic emissions compared with non-treated control rats following acute anoxia (Lopez-Gonzalez et al 1999). The protective action of melatonin in this study was again presumed to be due to melatonin's scavenging of free radicals that resulted from the ischaemia and associated anoxia.

In the kidney, melatonin attenuates the negative consequences of cisplatin that impose a severe oxidative stress on renal tissue. When rats were given a single intraperitoneal injection of 7 mg $kg^{-1}$ cisplatin, renal damage and dysfunction were readily apparent within 3–5 days (Hara et al 2001). Plasma creatinine and blood urea nitrogen (BUN) were increased in the rats treated with the drug. In the kidney, the GSH : GSSG ratio decreased, as did the activity of GSH-Px, while the levels of lipid peroxidation products rose after chemotherapy administration. Both the physiological impairments and the biochemical alterations were reversed in the rats given pharmacological doses of melatonin. Finally, melatonin prevented morphological damage to the proximal convoluted tubules, the epithelial cells that especially concentrate cisplatin from the blood, which was obvious in the kidneys of rats given cisplatin without melatonin. Since the melatonin metabolite 6-hydroxymelatonin was also found to be beneficial (although less so than melatonin) in reducing cisplatin toxicity, Hara et al (2001)

hypothesized that melatonin's ameliorative effects may have been partially a result of free radical scavenging by the metabolite. This is consistent with the antioxidant cascade as summarized above.

The cytotoxicity of cisplatin is also apparent in peripheral blood mononuclear cells. When these cells were exposed to cisplatin in-vitro, intracellular GSH levels were depleted, DNA fragmentation and laddering were apparent and the cells exhibited evidence of apoptosis. Each of these changes was prevented if melatonin was added to the medium concurrent with cisplatin (Hassan et al 1999). On the basis of these results, the authors concluded that melatonin might improve a patient's response to cisplatin therapy and perhaps prolong their survival.

Also, in reference to relationship of melatonin and cisplatin to cancer-cell proliferation, the publication of Futugami et al (2001) is of particular interest. Cisplatin is often used as a first-line treatment for highly aggressive ovarian cancer. Using a complicated experimental design where cisplatin was given either intermittently or sequentially, Futugami et al (2001) claimed that melatonin actually enhanced the sensitivity of HTOA and OVCAR (ovarian cancer cell lines) to cisplatin and proposed that melatonin may improve the efficacy of cisplatin as a treatment for ovarian cancer. Mechanistically, how melatonin altered the sensitivity of ovarian cancer cells to cisplatin was not clarified in this study, but the authors implied that the inhibition of cell proliferation probably involved the suppression of telomerase activity via a nuclear receptor for melatonin. Whereas the evidence supporting a nuclear receptor for melatonin is compelling for some cells (Garcia-Mauriño et al 2000), whether they were present in the cancer cells used in this study remains to be determined. If a nuclear receptor was involved in the sensitization of these ovarian cancer cells to cisplatin by melatonin, an antioxidative function of the indole is not necessarily precluded (e.g., melatonin may have altered the activity of antioxidative enzymes via a receptor-mediated process, as has been suggested for other studies) (Pablos et al 1997a).

The ability of melatonin to decrease the toxicity and increase the efficacy of cancer chemotherapeutic agents (Figure 5) has been exploited at the clinical level as well. Lissoni et al (1997) treated a total of 70 cancer patients (advanced non-small cell lung cancer or NSCLC) with a combination of cisplatin plus etoposide alone or the chemotherapeutic agents plus melatonin; 36 patients received the two chemotherapeutic agents while 34 were given them in combination with melatonin. Based on the complete and partial tumour response rate, melatonin enhanced the action of cisplatin + etoposide and also increased the 1-year survival rate. Additionally, the chemotherapies were better tolerated as indicated by significantly lower frequencies of myelosuppression, neuropathy and cachexia in the patients who received melatonin. It is important to note in regard to this study that whereas the improvement in the melatonin-treated cancer patients relative to those given the chemotherapies alone may not have been dramatic, the only patients included in the study were those with advanced end-stage cancers who were unable to tolerate very aggressive chemotherapies. Had melatonin (in combination with other therapies) been initiated at an earlier stage, it may have proven to be more effective in terms of the clinical status and survival of the patients.

This work was extended to a larger cohort of cancer patients by the same research group (Lissoni et al 1999a). The study included 250 patients with metastatic solid tumours (104 lung cancers; 77 breast cancers; 42 gastrointestinal cancers; 27 head and neck cancers) who were given a variety of different chemotherapies (cisplatin plus etoposide, gemicitabine, doxorubicin, mitoxantrone (mitozantrone), paclitaxel, 5-fluorouracil plus folinic acid, 5-fluorouracil + cisplatin) alone or in combination with melatonin. The objective tumour regression rate, as well as the 1-year survival rate, was improved by melatonin cotreatment and, moreover, melatonin significantly reduced the frequency of thrombocytopenia, neurotoxicity, cardiotoxicity, stomatitis and asthenia. Again, the patients included in this study were in the advanced stage of their disease where therapies of any type would probably have been minimally effective. Thus, the fact that melatonin treatment had any benefit seems exceptional. The utility of instituting melatonin treatment earlier in the progression of the cancer would seem reasonable, with the expectation of greater benefits. The findings of Lissoni and colleagues have recently been reviewed (Lissoni 2002). A shortcoming of these studies is that they were neither double blind nor placebo-controlled. Nevertheless, when considered in light of the animal data, which are supportive, the collective results make a rather strong case for the use of melatonin as a palliative agent in cancer patients.

*Epirubicin*

In another study, Lissoni et al (1999b) found that melatonin reversed persistent untreatable thrombocytopenia in 14 women with metastatic breast cancer who were being treated with epirubicin. Within 7 days of the inception of melatonin therapy, circulating platelet numbers had doubled (relative to the values before treatment). These findings, which are considered preliminary because of the small number of patients, do suggest that melatonin may be able to correct cancer-related thrombocytopenia, albeit that the mechanisms remain to be established. A later investigation indicated that the stimulatory effect of melatonin on platelet levels in cancer chemotherapy may not be a universal phenomenon; seemingly about 65% of these individuals respond to melatonin with augmented platelet counts (Lissoni et al 2001). The remaining 35% reportedly do increase their circulating platelet levels following treatment with melatonin, but only when it is combined with another pineal indole, 5-methoxytryptamine (Lissoni et al 2001).

These studies, as well as others by Lissoni et al (1997, 1999a) have been critiqued elsewhere (Blask 2001). While trials were not double blind or placebo-controlled, when the findings are considered in light of the animal studies summarized above, a strong case can be made for combining melatonin with routine chemotherapies for the purposes of improving the outcome and well-being of cancer patients given toxic chemotherapeutic agents.

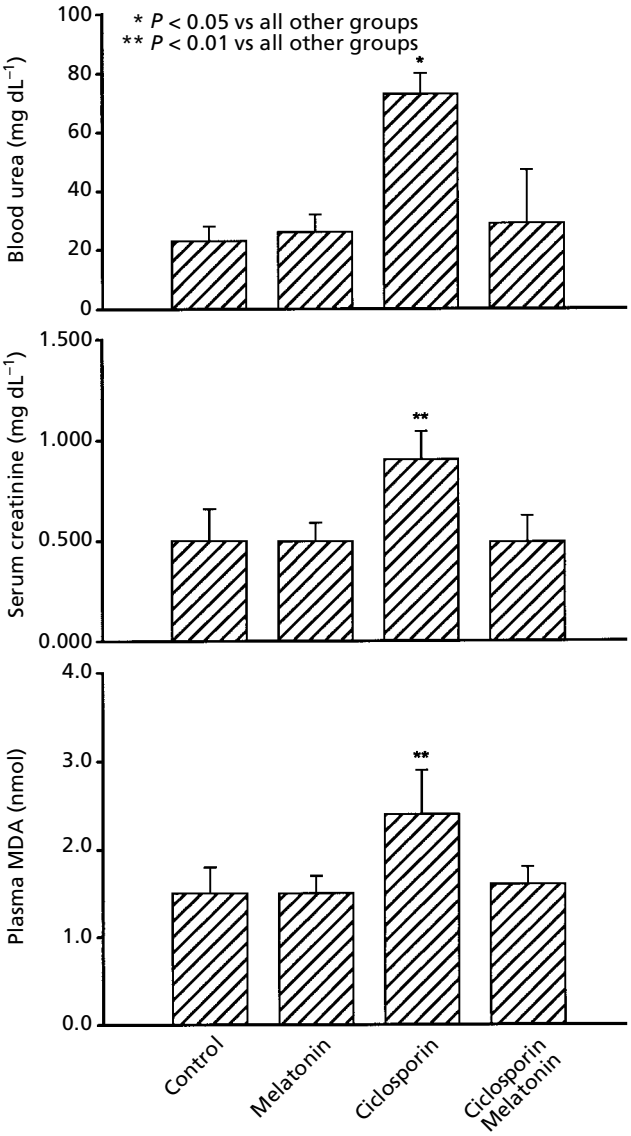


**Figure 6** Renal damage induced by ciclosporin leads to rises in circulating levels of blood urea, serum creatinine and plasma malondialdehyde (MDA) levels; the latter represents free-radical-mediated damaged lipids. Concurrent administration of melatonin returns each of these values to control levels.

*Bleomycin*

Another commonly used cancer chemotherapeutic agent, bleomycin, dose-dependently induces interstitial pulmonary fibrosis. Since bleomycin administration also increases the content of lipid peroxidation products, as well as changing the activity of antioxidative enzymes in bronchoalveolar fluid (BALF) and lung tissues, it has long been assumed (and in some cases shown) that antioxidants may protect against bleomycin toxicity. This information also served as the basis for an investigation into the actions of melatonin in possibly reducing exaggerated oxidative damage in the BALF of bleomycin-treated rats (Arslan et al 2002).

After 25 days of treatment of rats with bleomycin (10 mg $kg^{-1}$ i.p. twice daily), BALF was collected and analysed for total protein and activity of SOD, GSH-Px, CAT and lactate dehydrogenase (LDH). Each of these was increased after bleomycin treatment, as were the levels of GSSH and lipid-peroxidation products. Melatonin administration, also given intraperitoneally twice weekly at a 10 mg $kg^{-1}$ dose, markedly reduced all parameters indicative of oxidative stress. Additionally, melatonin ameliorated the increase in lung collagen (measured as hydroxyproline) caused by the chemotherapeutic agent. It was the judgment of the authors that, based on the outcome of their study, melatonin is a promising candidate as an agent to reduce bleomycin toxicity and prevent the associated interstitial pulmonary fibrosis (Arslan et al 2002).

*Cyatarabine*

Cytarabine is a mylosuppressive cytotoxic drug used in the treatment of cancer; this drug typically produces severe leucopenia, thrombocytopenia and anaemia (Calabresi & Chabner 1991). These effects also become apparent when the drug is injected into rats and the mechanisms of the myelosuppression, although not known with certainty, may partly involve an inhibition of DNA synthesis and repair. Since melatonin had been reported to protect against the cytotoxicity of other chemotherapeutic agents, Anwar et al (1998) tested melatonin's efficacy in reducing the toxicity of cytarabine. Rats given the drug alone exhibited many signs of bone-marrow damage, including reduced peripheral red blood cell (RBC) counts, lowered total leucocyte counts and a reduced number of platelets. Additionally, cytarabine decreased serum total proteins and albumin and increased the albumin : globulin ratio. The authors reported that each of the changes associated with cytarabine administration were prevented in the rats given melatonin after the injection of the drug. Whether the beneficial effects of melatonin in this study had any relationship to the antioxidant properties of the indole was not determined and the authors presented no evidence of exaggerated free radical generation in cytarabine-treated rats.

*Gentamicin*

Aminoglycoside antibiotics are commonly used for the treatment of Gram-negative bacterial infections. Perhaps the most widely used drug in this category is gentamicin. Major negative effects of this drug involve the kidney, and include severe morphological damage and functional deficits (Simmons et al 1980); in the worst cases, acute renal failure is a consequence (Humes & Weinberg 1986). The toxicity of gentamicin at the level of the kidney seems to relate to the fact that the proximal convoluted tubules exhibit a predilection for uptake and retention of the drug, which is followed by the generation of destructive reactive oxygen species in these cells (Walker & Shah 1988).

Several research groups, on the basis of this information, surmised that melatonin may attenuate gentamicin-induced nephrotoxicity and pathology and all three groups found their suppositions to be correct. Shifow et al (2000) treated rats for 8 days with gentamicin (80 mg

daily i.p.); the rats were given melatonin (5 mg $kg^{-1}$ p.o.) for 3 days before, and daily during gentamicin treatment. The aminoglycoside induced a variety of negative effects indicating renal damage. Thus, these rats had increased blood urea and serum creatinine levels, reduced creatinine clearance, rises in lipid peroxidation products in both the serum and kidney and increased urinary excretion of *N*-acetyl-$\beta$-D-glucosaminidase, glucose and protein. When melatonin and gentamicin treatment were combined, each of these values was comparable with that in the control rats. The authors concluded with the suggestion that melatonin may play an important role in nephroprotection generally and specifically with regard to reducing gentamicin toxicity.

Using some of the same parameters as well as others, Özbek et al (2000) and Sener et al (2002) also found that melatonin alleviated the kidney of the damaging effects of gentamicin. According to Özbek et al (2000), melatonin prevented hyperproteinuria, the marked increase in renal lipid peroxides, the reduction in renal antioxidative enzyme activity (i.e., SOD, GSH-Px and CAT) and the massive tubular necrosis that followed gentamicin administration. Likewise, using the same functional parameters, as well as others indicating elevated oxidative stress (e.g., increased oxidized renal protein and myloperoxidase activity), Sener et al (2002) found that melatonin, once again, overcame the negative aspects of gentamicin at the level of the kidney. This group also presented histological evidence of protection by melatonin in the renal cortex. Considering the reduced oxidative damage due to melatonin treatment, both groups attributed melatonin's protective actions to its direct and indirect antioxidant activity.

In addition to the kidney, aminoglycosides induce ototoxicity (Henley & Rybak 1993). Lopez-Gonzalez et al (2000a) used either gentamicin or tobramycin to cause distortions in the otoacoustic emissions in rats. In this in-vivo study, melatonin administered in combination with each aminoglycoside prevented the toxicity of the drugs. In an accompanying in-vitro study, this group reported that melatonin does not interfere with the antibiotic capability of the aminoglycosides; the doses of melatonin in these studies ranged from physiological to pharmacological. As has now become a theme in these reports, melatonin seems to reduce the toxicity of a variety of drugs without decreasing their efficacy (and in some cases increasing their potency).

*Ciclosporin*

Ciclosporin is an immunosuppressive agent that is useful in individuals who have received an organ transplant and in the treatment of autoimmune diseases (Wolf et al 1997). It does, however, have significant side effects that involve primarily the kidney and the liver. While the mechanisms that account for the toxicity of this drug are unknown, the experimental data suggest that free radical generation at these sites may be responsible for the tissue damage (Wolf et al 1997).

Considering this, Vijay Kumar et al (1999) speculated that melatonin would likely protect against the nephropathy caused by ciclosporin. In their study, rats were given either ciclosporin alone or in combination with melatonin. Melatonin, taken orally, was given for 3 days in advance of ciclosporin administration and for an additional 2-week period in combination with ciclosporin. Of the many functional parameters assessed, melatonin prevented the increase in blood urea, diminished the fall in creatinine clearance and curtailed the rise in plasma levels of lipid peroxidation products, all of which were induced by ciclosporin (Figure 6). Additionally, melatonin prevented ciclosporin-induced morphological damage to the renal cortex. The authors surmised that it was melatonin's antioxidative capacity that protected the kidney from ciclosporin-induced damage, although the evidence supporting this was indirect.

The liver also exhibits damage after ciclosporin treatment. When rats were given ciclosporin for 2 weeks, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase ($\gamma$GT) were elevated, with the responses being attenuated in those rats given melatonin (Kwok & Mun 2000). Likewise, hepatic oxidized lipid products, which were increased as a consequence of ciclosporin administration, were decreased in melatonin-injected rats. There were some parameters (e.g., serum alkaline phosphatase) which were increased as a result of ciclosporin injection but that were not reversed by melatonin. In sum, however, the authors concluded that melatonin provides significant protection at the level of the liver when this organ is functionally and biochemically compromised by ciclosporin (Kwok & Mun 2000).

*Indometacin*

A prominent side effect of the use of indometacin, a non-steroidal anti-inflammatory drug (NSAID), is ulcerative lesions of the gastrointestinal tract. The mechanism by which indometacin induces ulcers is not totally apparent but seems to involve inhibition of prostaglandin synthesis and oxygen radical generation (Mahmud et al 1996). Alarcon de la Lastra et al (1999) used a rat model of indometacin toxicity to determine the potential protective actions of melatonin against lesions to the gastric mucosa caused by a single oral dose of indometacin (20 mg $kg^{-1}$); melatonin was injected intraperitoneally at doses of either 0.25, 0.5, or 1 mg $kg^{-1}$) to groups of rats 30 min before gastric indometacin loading. The investigative end-points measured included an assessment of leucocyte infiltration (as indicated by myloperoxidase (MPO) activity) into the stomach mucosa, measurement of lipid peroxidation products, GSH-Px activity and the ulcer index (total $mm^2$ of damaged mucosa). Melatonin administration in advance of indometacin instillation reduced the ulcer index and lowered the level of thiobarbituric acid reactive substances (TBARS or lipid peroxidation products). Additionally, melatonin restored the depressed levels of GSH-Px but did not reduce MPO activity, suggesting that the degree of leucocyte infiltration was not modified by melatonin. Despite the failure of melatonin to reduce MPO activity, the amount of oxidatively damaged tissue was clearly reduced and the authors attributed melatonin's protective actions to the

indole's radical scavenging activity and possibly to other unidentified mechanisms.

Othman et al (2001) conducted a similar study in which indometacin was used to induce both gastric damage and testicular oxidative stress. In this experiment, indometacin was given intragastrically at a dose of 20 or 30 mg $kg^{-1}$ and, as in the previous study, melatonin was administered 30 min in advance of indometacin; melatonin was given intragastrically as well. In rats given melatonin only, all measured parameters were equivalent to those of the controls at 4 h after treatment. Gastric damage, evaluated as the total extent ($mm^2$) of mucosal erosion, was highly and significantly increased after indometacin alone and was reduced by 80% in the melatonin-treated rats. Likewise, lipid peroxidation and LDH in the liver were augmented following drug treatment, with the former of these being reversed by melatonin. Indometacin also provoked a reduction in Cu/Zn-SOD and mitochondrial Mn-SOD activity and GSH levels, all of which were reversed by melatonin. In the testes as well, melatonin reduced the toxic changes induced by indometacin. Othman et al (2001) conclude that melatonin co-treatment would seem to be a reasonable co-treatment when using indometacin as well as other free-radical-generating drugs.

Some light was shed on the mechanisms by which melatonin limits indometacin toxicity in the recent studies of Brzozowska et al (2002). Using rats, indometacin-induced damage at the level of the stomach was shown to be attenuated by melatonin; however, the beneficial effect of melatonin was blocked when the membrane melatonin receptor antagonist luzindole was co-administered. The obvious implication is that receptor-mediated actions of melatonin account for its ability to reduce indometacin toxicity in the gastric mucosa. Antioxidative processes, however, may still have played a role in melatonin's protective actions since the ability of melatonin to stimulate GSH synthesis and recycling, as well as the activity of antioxidative enzymes, are presumed to involve melatonin's interaction with receptors.

*Acetylsalicylic acid*

Another NSAID that is noted for its significant gastric toxicity is acetylsalicylic acid (ASA or aspirin). The negative consequences of acetylsalicylic acid are believed to be a result of inhibition of prostaglandin synthesis and a direct irritant action on the mucosa, as well as other yet undefined processes (Inauen et al 1998). Melatonin has been compared with two well known anti-ulcer drugs, omeprazole and famotidine, in terms of their ability to protect against acetylsalicylic-acid-induced gastric mucosal damage (Sener-Muratoglu et al 2001). Besides the degree of gastric ulcerogenesis caused by acetylsalicylic acid in the rat, this group measured a variety of parameters indicative of oxidative damage including oxidized lipids, GSH levels and myloperoxidase activity. In this in-vivo study, not only did melatonin prevent acetylsalicylic acid-induced damage in the stomach, but also, according to the authors, it was more effective than either omeprazole or famotidine in doing so. The ability of melatonin to preserve the integrity of the mucosal epithelium that is normally aggravated by salicylate treatment is not trivial considering the widespread use of acetylsalicylic acid and the frequency with which it induces serious gastrointestinal complications. From this study the authors were unable to discern the mechanisms of melatonin's protective actions but they presumed that the antioxidant properties of the indole contributed to its protective actions. There is, however, one report in which melatonin was found to be ineffective in protecting rat gastric mucosa from acidified acetylsalicylic acid, even though this group reported melatonin-induced suppression of gastric ulcers resulting from a number of other treatments (Brzozowski et al 1997).

*Ranitidine and omeprazole*

Several studies have compared melatonin's efficacy in protecting against stress-induced gastric ulceration with widely used anti-ulcer medication such as ranitidine and omeprazole (Bandyopadhyay et al 2000, 2001, 2002). Ranitidine blocks histamine-stimulated acid secretion in the stomach while omeprazole, a substituted benzimidazole, completely interrupts acid secretion. Both drugs however, have side effects including, among others, headache, dizziness, diarrhoea and skin rashes. Bandyopadhyay et al (2000) initially used rats subjected to either restraint–cold stress or treated with indometacin to compare the relative ability of melatonin, ranitidine or omeprazole in reducing signs of oxidative damage to the stomach. Melatonin was found to dose-dependently reduce gastric mucosal damage in these ulcerative models and to be more effective than ranitidine but less efficient than omeprazole in lowering the incidence of ulcers. When compared with other antioxidants, melatonin was better than glutathione and equipotent to vitamin E in protecting the stomach from mucosal damage caused by cold–restraint stress or indometacin administration.

Importantly, in a follow-up study, this group showed that when melatonin treatment was combined with either ranitidine or omeprazole the drugs were much more efficient in reducing stress-mediated mucosal breakdown in the stomach. Thus, co-treatment of rats with melatonin and either anti-ulcer drug protected the gastric mucosa from ulcers at doses where either drug alone had no protective effect. This is important since by reducing the dose of either ranitidine or omeprazole, the side effects also would likely be eliminated or significantly suppressed.

Mechanistically, in these ulcer models, melatonin was shown to be protective by virtue of its ability to scavenge $^{\cdot}OH$ (Bandyopadhyay et al 2001), which the authors had previously shown to be involved in the generation of experimental gastric ulcers (Das et al 1997). However, it seems likely that, in addition to its direct scavenging actions, melatonin has other indirect (antioxidative and otherwise) means by which it reduces damage to the lining of the stomach.

*Isoniazid*

Isoniazid is a first-line drug for the treatment of tuberculosis but there are major problems with its usage including the rapid development of resistance to the drug and adverse

reactions in many patients. In an in-vitro study, Wiid et al (1999) incubated *Mycobacterium bovis* and strains of drug-sensitive and drug-insensitive *M. tuberculosis* with and without melatonin added to the medium. In this study melatonin enhanced the activity of isoniazid roughly 3.5 fold; the concentrations of melatonin used were considered pharmacological since they were higher than normal blood levels of the indole. Interestingly, at doses where neither drug alone exerted a significant inhibition of mycobacterial growth, the combined agents had a substantial inhibitory effect. If these relationships would hold in-vivo, isoniazid could be given it a much lower dose with increased efficacy and reduced toxicity.

*Iron and erythropoietin*

In individuals suffering from anaemia with chronic renal failure, the treatment often includes intravenous iron (Fe) and recombinant human erythropoietin (rHuEPO) (MacDougall et al 1989). Both of these agents have side effects which likely involve free radical damage since iron, via the Fenton reaction, generates $^{\bullet}OH$, and erythropoietin enhances $O_2^{-\bullet}$ production by leucocytes and leads to oxidative damage in erythrocytes (Zachee et al 1993).

This being the case, Herrera et al (2001) examined the ability of melatonin, given orally, to suppress the oxidative damage in chronic haemodialysis patients treated with Fe+rHuEPO. Nine patients (5 male, 4 female) were followed in this study and the oxidative end-points included plasma malondialdehyde, erythrocyte GSH and CAT activity; blood samples were obtained before treatment and at 1, 3 and 24 h after the administration of Fe (100 mg Fe saccharate, i.v., in 1 h) or rHuEPO (4000 U, i.v.). Melatonin (0.3 mg $kg^{-1}$) or placebo was given 60 min in advance of the drug treatment. Increased oxidative stress was apparent in patients with end-stage renal failure with the degree of molecular damage increased in the individuals who received Fe or rHuEPO. Melatonin administration prevented the oxidative stress associated with both Fe and rHuEPO treatment and had no apparent side effects. Whether this protective effect of melatonin improved the well-being or prolonged survival of these patients was not determined, but the authors felt that melatonin therapy in individuals with chronic renal failure on haemodialysis should be a consideration.

*Phenobarbital*

A large amount of experimental work suggesting that melatonin is able to suppress neuronal excitability led Molina-Carballo et al (1997) to use melatonin as an adjunctive treatment in a child with myoclonic epilepsy. The patient, a 29-month-old female, had been treated with a variety of different anti-epileptics. Despite these treatments, the seizures continued and the child exhibited severe neurological and psychomotor deterioration; before melatonin was given she was semi-comatose. Within 1 month of beginning high pharmacological doses of melatonin (100 mg daily) in combination with an ineffective dose of phenobarbital, the seizures abated. As the melatonin dose was reduced over time, the seizures reappeared and were again suppressed when melatonin doses were increased. The specific mechanisms whereby melatonin reduced seizure activity in this child remains unknown but may include an inhibitory action of melatonin on glutamate receptors and a potentiation of the $GABA_A$-benzodiazepine receptor (Acuña-Castroviejo et al 1995). For a more thorough discussion of the possible utility of melatonin in the treatment of neurological disorders in children, the reader is referred to review articles by Muñoz-Hoyos et al (1998) and Fauteck et al (1999).

*Carbamazepine*

In animal studies, melatonin seems to have been effective in inhibiting epileptic activity induced by electroconvulsive shock. Large doses of melatonin (50 mg $kg^{-1}$, 60 min before test) were shown to elevate the electroconvulsive threshold of mice, with the effect being reversed by aminophylline, picrotoxin or bicuculline (Borowicz et al 1999). From their studies using other pharmacological agents, the authors theorized that melatonin's anti-electroshock efficacy may involve neural adenosine and GABA. At a lower dose (25 mg $kg^{-1}$), melatonin also potentiated the anticonvulsive activity of carbamazepine and phenobarbital. The authors mentioned that melatonin, although it was found to have anticonvulsant activity and was devoid of adverse motor effects, may not be useful because of the memory deficits it seemed to cause in the mice. Alterations in memory due to melatonin treatment have, to these authors' knowledge, never been reported in any other animal or human studies so this unusual observation should be interpreted with caution.

Besides its use as an anti-epileptic, carbamazepine is also used in trigeminal neuralgia, alcohol withdrawal syndrome and affective disorders. Long-term treatment with carbamazepine may result in genotoxic effects as evidenced by DNA damage in peripheral leucocytes (Flejter et al 1989). When human lymphocytes were treated in-vitro with carbamazepine (4–12 $\mu$g $mL^{-1}$), chromosomal aberrations and sister chromatid exchanges increased, but when the cells were pre-treated with melatonin (0.5 mM) the incidence of genetic damage was significantly attenuated (Awara et al 1998). The authors concluded that melatonin may be an effective co-therapy to reduce the DNA damaging potential of carbamazepine, and the discussion of the findings indicated that these workers attributed melatonin's productive actions to its antioxidant capability.

*Haloperidol*

Haloperidol, a dopamine-receptor antagonist, is used in a variety of clinical situations for the treatment of psychoses and agitation. This drug is concentration-dependently cytotoxic in mouse clonal hippocampal HT22 cells; the basis for the toxicity is believed to be a result of induced oxidative stress. In a comparative study of the potential protective actions of antioxidants against haloperidol-induced neuronal damage, Post et al (1998) found that while both melatonin and *N*-acetylserotonin protected the

cells against haloperidol, vitamin E provided greater protection. The supposition was that the antioxidants provided protection via two means (i.e., they blocked lipid peroxidation and reduced haloperidol-induced activation of NF-$\kappa$B). The workers believe antioxidants, especially vitamin E, could be used to protect against haloperidol toxicity.

These observations are of interest and consistent with other findings. Thus, as an in-vitro antioxidant vitamin E seems often to be more effective than is melatonin, while in-vivo this is often reversed. It is possible that melatonin fares better in the organism because it is more widely distributed in the body and within cells. This may be especially true in the brain, since vitamin E, unlike melatonin, crosses the blood–brain barrier with difficulty. Thus, what Post et al (1998) described in their in-vitro studies concerning the high efficacy of vitamin E against haloperidol cytotoxicity may not be true in in-vivo situations.

Melatonin's protective effect against haloperidol was recently documented in patients in a thorough report by Shamir et al (2001). In a double-blind, placebo-controlled, crossover study, this group found that melatonin (10 mg daily), given for only 6 weeks to patients exhibiting tardive dyskinesia due to antipsychotic drug treatment, markedly reduced the movement disorders as assessed by the Abnormal Involuntary Movement Scale (AIMS). The difference, relative to the placebo-treated controls, was highly statistically significant and no adverse side effects of melatonin therapy were noted. Besides tardive dyskinesia associated with haloperidol (13 patients), this study also showed that the abnormal involuntary movements induced by other antipsychotics (i.e., chlorpromazine (4 patients), perphenazine (3 patients) and zuclopenthixol (2 patients)) were also reduced when the patients were given melatonin. While the sample size was small and the treatments varied, the universal response of the patients lends support to the authors' suggestion that melatonin may be an effective treatment to attenuate tardive dyskinesia in patients being treated with antipsychotic mediations.

*Caposide-50*

Caposide-50 (C-50) is an antihypertensive drug that is used to treat stage II essential hypertension. While C-50 alone was found to lower systolic, diastolic and mean arterial blood pressure, when it was combined with concurrent melatonin treatment the reductions in these haemodynamic parameters was further increased (Zaslavskaia et al 2000). Melatonin was not given as the sole treatment to any of the patients. The findings are generally consistent with the antihypertensive effects of melatonin in animals but the mechanism whereby melatonin would enhance the actions of C-50 remain unknown.

*Morphine*

Melatonin by itself seems not to alter the response to painful stimuli but when combined with either diazepam or morphine (or both), the indole was shown to increase the antinociceptive effect of these drugs. In the study in question, Pang et al (2001) used the formalin test in mice; this agent typically elicits two phases of the pain response. When melatonin (20 mg $kg^{-1}$) was injected intraperitoneally 15 min before the combined treatment with diazepam (1 mg $kg^{-1}$) and morphine (50 mg $kg^{-1}$), there was a synergistic analgesic effect in the first- and second-phase response. Although several possible explanations for the analgesic-enhancing effects of melatonin were discussed, all were highly theoretical and left the impression that none were favoured over any other. Clearly, the mechanisms remain enigmatic.

*Cyclophosphamide*

The DNA damage induced by cyclophosphamide in Chinese hamster ovary cells was reportedly reduced by the addition of melatonin to the medium in which the cells were grown (da Salvia et al 1999). The protection by melatonin was manifested as reductions in both chromosomal aberrations and sister chromatid exchanges. The mutagenicity of cyclophosphamide, an alkylating agent, is related to the formation of the cytotoxic metabolite phosphoramide mustard which ultimately induces DNA cross-links and strand lesions (Hengstler et al 1997). Melatonin in this model may have modified induced chromosomal damage either by altering cyclophosphamide metabolism or through its function as an antioxidant. In another section of their report the authors produced circumstantial evidence that melatonin may have oxidative activity, but they provided no biochemical or morphological documentation of this. Melatonin has been widely tested for oxidant actions; these tests, when conducted in-vivo, have routinely failed to show that melatonin exaggerates oxidative stress.

*L-cysteine*

L-cysteine is classified as an orphan drug that has potential utility in the treatment of erythropoietic protoporphyria. When mouse-brain homogenates were exposed to concentrations of L-cysteine in the range 0.05–2.0 mM, it increased the levels of lipid peroxidation products in a concentration-dependent manner (Yamamoto & Tang 1996c). The addition of melatonin (2 mM) suppressed or completely abolished the oxidative breakdown of lipids in the neural homogenates. In light of melatonin's ability to retard the generation of products of lipid peroxidation, Yamamoto & Tang (1996c) presumed the protective effect of melatonin was related to its free radical scavenging activity. The 2 mM concentration of melatonin was the only dose tested so a minimal effective concentration was not established.

## Melatonin: pharmacology vs physiology

In virtually all the studies summarized above, what was assumed to be pharmacological levels of melatonin were used to combat drug toxicity. On the basis of this, a number of workers have implied that melatonin is not relevant as a physiological antioxidant. To draw this conclusion based on the results of the studies performed is, however, erroneous. Oxidative stress develops because the endogenous antioxidative defence system in-toto is unable to cope with the massive number of reactants generated within a cell.

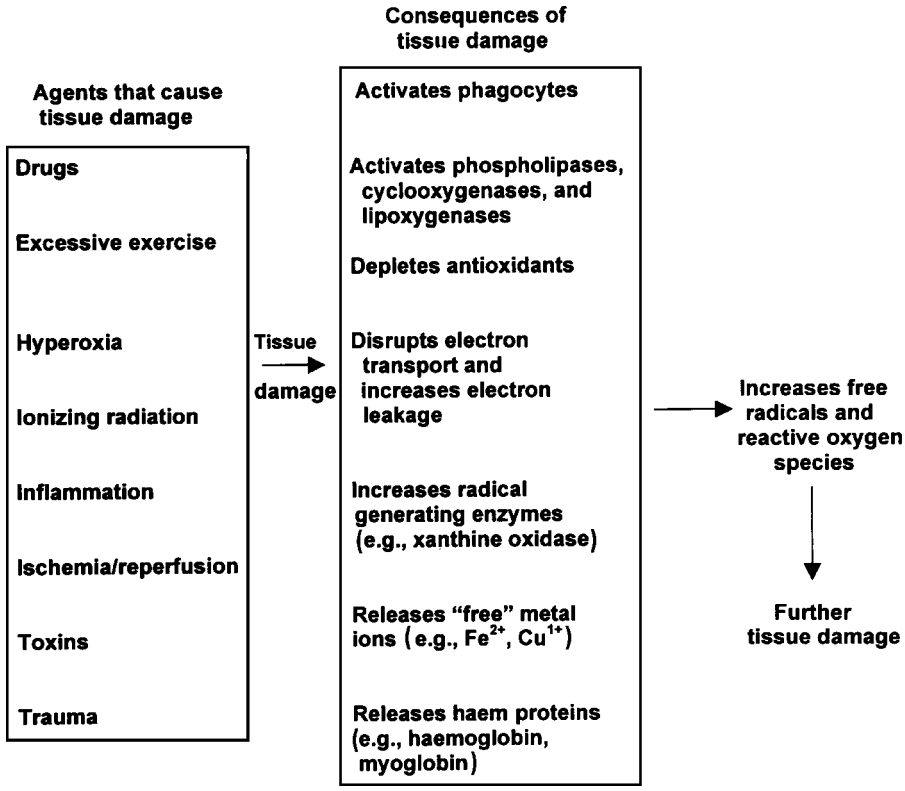


**Figure 7** A summary of the agents and processes (left) which cause tissue damage via free radical mechanisms. Besides drugs, summarized in the current review, many other situations lead to extensive molecular and tissue damage. Although beyond the scope of this review, besides preventing the toxic damage induced by drugs, melatonin also has been shown to reduce the damage caused by all the processes listed in the left-hand box. These data are summarized in some of the review articles cited in this paper.

When organisms are exposed to drugs, such as those described above, or other free radical generating agents or processes (Figure 7), the number of reactants produced overwhelms the capacity of the system to defend itself. In these situations supraphysiological (pharmacological) levels of an antioxidant must be given to prevent plundering by the large number of toxic reactants that are induced.

A second argument that has been levelled is that endogenous levels of melatonin are so low it would not compete with antioxidants that are present in higher concentrations. Commonly, the definition of what constitutes a physiological level of melatonin relies on its concentration in the blood, which is typically in the range 0.5–1.0 nM (Reiter 1991). However, in other body fluids melatonin concentrations are sometimes orders of magnitude higher (Figure 8). In the bile, for example, levels range up to 4000 pg $mL^{-1}$ (Tan et al 1999a) compared with maximal blood levels of around 250 pg $mL^{-1}$ (Reiter 1991; Arendt 1988, 1993a, b). Likewise, within the cerebrospinal fluid, recent studies have shown that melatonin concentrations can be very high, up to roughly 90 nM (Kanematsu et al 1989; Skinner & Malpaux 2000; Tricoice et al 2001). Within some cells and subcellular compartments also, melatonin levels exceed those in the blood many fold (Menendez-Pelaez & Reiter 1993; Menendez-Pelaez et al 1993; Martin et al 2000b). This is especially important since free radicals and related reactants often have very short half-lives (a fraction of a second) and travel ultra-short distances (a couple of molecular diameters) before they inflict their damage (Borg 1999). For an antioxidant to neutralize a toxic reactant before it mutilates a bystander molecule, it must be very near to where the reactant was generated. Thus, the distribution of an antioxidant within a cell, as determined by its unique solubility and other factors, is critical to its ability to defend against free radicals. Unlike vitamin E, the lipid solubility of which limits its distribution within cells, melatonin seems to be ubiquitously distributed, albeit unevenly, in subcellular compartments since it is amphiphilic.

Considering the highly divergent concentrations of melatonin in different fluids and subcellular compartments, many of which seem to be considerably higher than those routinely measured in the blood, comments concerning what constitutes a physiological level of melatonin are meaningless unless they are defined in terms of a specific compartment. Thus, while the majority of studies summarized in the current review provided doses of melatonin that surely caused circulating levels of the indole to exceed those normally found in the blood, whether this was true for all compartments remains unknown. However, considering the ease with which melatonin manoeuvres between compartments, it can be assumed that melatonin was

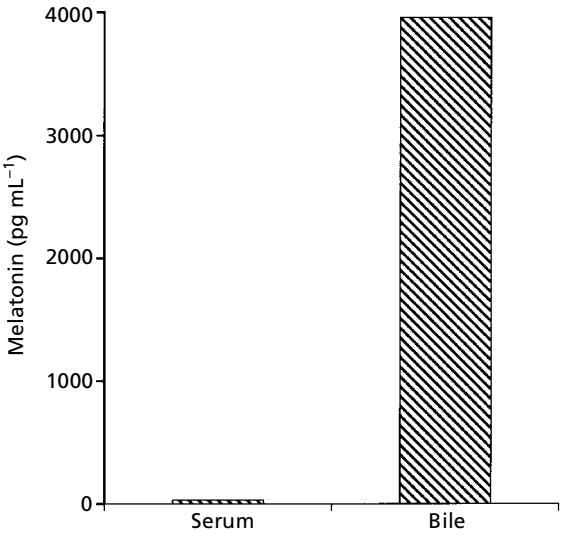


**Figure 8** Simultaneous mean concentrations of melatonin in the blood and bile of mammals, including man. These observations emphasize the fact that melatonin in different compartments is not in equilibrium. In the third ventricular cerebrospinal fluid melatonin levels are equivalent to those in the bile. Thus, when referring to physiological levels of melatonin they must be defined relative to a specific body fluid or subcellular compartment.

at pharmacological concentrations at all sites. Further investigations should, nevertheless, encompass measurements to confirm this supposition.

Another important factor in melatonin's role as an antioxidant within a vertebrate organism is its ability to transverse established morphophysiological barriers. Thus, vitamin E is a less important antioxidant in the brain since its concentration in this organ is low due to its inability to readily cross the blood–brain barrier. Conversely, melatonin penetrates this barrier easily (Yellon & Longo 1987; McMillen & Nowak 1989) and within minutes after its peripheral administration, brain cell concentrations of melatonin exceed those in the blood (Menendez-Pelaez & Reiter 1993; Menendez-Pelaez et al 1993). These same general rules apply to the placenta. Vitamin E is not a particularly effective antioxidant in reducing free radical toxicity in the fetus because of its limited ability to penetrate the placental barrier. Again, melatonin's transfer to the fetal blood is minimally impeded at the level of the placenta (Okatani et al 1998), and giving melatonin to a pregnant rat has already been shown to be effective in reducing free radical damage in fetuses (Wakatsuki et al 1999, 2001).

Clearly, discussions of physiological and pharmacological actions of antioxidants must take into account the very important matter of how well the agent distributes in the organism and its specific concentration at the site of free radical generation.

The ability of an antioxidant to interact with other detoxifying agents also determines its efficacy. For example, the relationship of vitamin E, vitamin C and glutathione in terms of their recycling of each other between the reduced and oxidized forms are critical to their efficacies as free radical scavengers. While less is known concerning the potential recycling of melatonin, it has been shown that when melatonin is concurrently used with vitamins E or C, there is a clear synergistic effect in terms of reducing oxidative damage (Gitto et al 2001b). Thus, the protection afforded by the combined treatment is greater than the sum of the two benefits that would result had the antioxidants been given individually. The mechanisms of these synergistic interactions of melatonin with these classical vitamin antioxidants remain unknown.

As noted above, not only is melatonin a direct free radical scavenger (Hardeland et al 1993, 1995; Reiter 1997; Tan et al 2002), but also some of the resulting metabolites are as well (Tan et al 2000b, 2001). This, in effect, increases the apparent concentration of melatonin by allowing it to scavenge several reactants per melatonin molecule as opposed to only scavenging one. Finally, the use of melatonin as a drug, regardless of whether it is in physiological or pharmacological doses, should be considered in view of its high efficacy.

## Use of melatonin: theoretical considerations

Notwithstanding a couple of reports that may indicate otherwise, the vast majority of the studies summarized above show that melatonin reduces the toxicity of a wide variety of currently used drugs and that in some cases the efficacy of these agents is also enhanced. These findings would seem to justify the consideration of using melatonin as a co-treatment with these medications. Certainly, the negative side effects of many drugs are substantial and minimizing these would be important. Additionally, however, for those drugs where melatonin lowers their toxicity, the dose could presumably be increased to make the medication more efficacious (e.g., cancer chemotherapeutic agents (Figure 5)).

It is somewhat surprising that melatonin ameliorates the toxic reactions of such a wide variety of drugs. Because of this, it seems unlikely that melatonin is only functioning as a direct free radical scavenger and indirect antioxidant in these situations. Rather, melatonin, in addition to its ability to detoxify radicals and related reactants (Reiter et al 2000b; Tan et al 2000b, 2002), presumably has some basic actions within subcellular organelles which improve the ability of the cells (and thereby the tissues and organs) to function more efficiently and resist mutilation by toxic reactants. Indeed, melatonin seems to prepare cells to cope with disastrous situations.

Assuming that physiological levels of melatonin have a function similar to those of the pharmacological concentrations used in many of the reports summarized herein, it may be relevant that endogenous melatonin levels change substantially throughout a lifetime and also there are sometimes substantial differences in the quantity of melatonin produced by different individuals (judged by their maximal blood concentration) (Arendt 1993a). The pertinent question that then arises is, are individuals with low endogenous melatonin production more susceptible to the negative side effects of drugs than are those patients who normally produce large amounts of the indole? This can

also be extrapolated to other patients where melatonin production is normally compromised. As humans (and animals) age, endogenous melatonin production wanes such that in the elderly, blood melatonin concentrations are often a fraction of what they are in young individuals (Reiter 1992, 1997; Arendt 1993b). The corollary question is, are the elderly more vulnerable in terms of the negative consequences of drugs because of their lower melatonin levels? Certainly, the elderly generally use more of the medications mentioned above than do younger individuals. Thus, should at least the older generation receive supplemental melatonin not only to restore youthful levels of this seemingly beneficial agent, but also to help them resist the onslaught of destructive reactions that accompany the taking of many medications? It would seem justified to more extensively test these possibilities in clinical trials, especially given that the studies that have been performed have proven to be successful (Lissoni et al 1997, 1999a, b, 2001; Brusco et al 1998, 2000; Cohen-Mansfield et al 2000; Fulia et al 2001; Gitto et al 2001a; Herrera et al 2001; Shamir et al 2001).

Besides its concurrent use with drugs, where its beneficial effects have been documented, would melatonin be useful as a prophylactic agent? If, in fact, melatonin has actions at the molecular level which optimize functions (e.g., mitochondrial respiration, ATP production, gene transcription and translation, protein synthesis, etc.), it could maintain the cell in a physiological state that would aid it in defending itself against destructive processes, not unlike optimal nutrition does in helping to resist disease. Thus, supplemental melatonin late in life could, theoretically at least, maintain a higher quality of life and defer diseases that prey on weakened defences such as a compromised immune system, depressed antioxidant reserve, sluggish ATP generation, etc. Such issues should be the subject of additional thought and increased investigation in a world where life span, and therefore the likelihood of developing age-related diseases, is measurably increasing.

### Concluding remarks

Research related to the antioxidant and related properties of melatonin has progressed at a feverish pace in the last decade. Almost daily, publications appear which examine the efficiency of melatonin in abating the toxic reactions of drugs and other processes that generate free radicals and associated reactants (e.g., ischaemia–reperfusion injury, toxin exposure, etc.). Although these latter findings were beyond the scope of this review, they are germane to what is discussed here and can easily be found by conducting a computer search of the literature.

What is obvious from the collective results of many investigations is that the protective actions of melatonin at the cellular level are not trivial. In view of the strikingly beneficial actions of the indole, as well as its very low level of toxicity (Nordlund & Lerner 1976; Wright et al 1986; Terzolo et al 1990; Kane et al 1994; Brusco et al 1998; Jahnke et al 1999; Cohen-Mansfield et al 2000; de Lourdes et al 2000; Jan et al 2000), it would seem imperative to make more widespread use of this molecule in medicine. Besides its high safety margin, melatonin is inexpensive to produce in a pharmacologically pure form. What melatonin has working against it is the fact that it is a naturally occurring, non-patentable molecule. Thus, pharmaceutical interest in this agent per-se is low, despite its low toxicity and high efficacy.

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