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MINI REVIEW

Cardiovascular diseases: protective effects of melatonin

Abstract: This brief review considers some of the cardiac diseases and conditions where free radicals and related reactants are believed to be causative. The report also describes the beneficial actions of melatonin against oxidative cardiovascular disorders. Based on the data available, melatonin seems to have cardioprotective properties via its direct free radical scavenger and its indirect antioxidant activity. Melatonin efficiently interacts with various reactive oxygen and reactive nitrogen species (receptor independent actions) and it also upregulates antioxidant enzymes and downregulates pro-oxidant enzymes (receptor-dependent actions). Moreover, melatonin enters all cells and subcellular compartments and crosses morphophysiologic barriers. These findings have implications for the protective effects of melatonin against cardiac diseases induced by oxidative stress. Melatonin attenuates molecular and cellular damages resulting from cardiac ischemia/reperfusion in which destructive free radicals are involved. Anti-inflammatory and antioxidative properties of melatonin are also involved in the protection against a chronic vascular disease, atherosclerosis. The administration of melatonin, as a result of its antioxidant features, has been reported to reduce hypertension and cardiotoxicity induced by clinically used drugs. The results described herein help to clarify the beneficial effects of melatonin against these conditions and define the potential clinical applicability of melatonin in cardiovascular diseases.

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Introduction

In economically well-developed countries of the world, cardiovascular diseases are the major cause of disability and mortality [1]. Ischemia/reperfusion (I/R), atherosclerosis, hypertension, and cardiotoxicity induced by drugs are some of the most important situations/disease processes where free radicals impact cardiac physiology [2–5].

Reactive oxygen species (ROS) play an important role in the pathogenesis of cardiac I/R injury. The mechanism for the enhanced ROS generation as well as cellular and subcellular targets of ROS attack is not totally known. One of the main sources of ROS in cardiomyocytes during ischemia and early reperfusion may be a faltering electron transport chain in mitochondria [6–10].

The fundamental processes underlying the development of vascular disease, such as atherosclerosis, have their origins in an initial insult to the vessel wall. Such an insult may arise from mechanical disruption or it can result from biologic causes, such as hypercholesterolemia, diabetes, increased concentrations of plasma homocysteine, infectious agents and an excess of free radicals. There is general agreement that ROS play a vital role in the pathogenesis of coronary atherosclerosis and its complications [11, 12].

Hypertension is recognized as a multi-factorial situation resulting from the effect of a combination of environmental and genetic factors. Some of the factors that contribute to hypertension include excess dietary salt or alcohol intake, stress, age, genetics and a family history, obesity, physical inactivity, as well as a high saturated fat diet [13]. Increased oxidative stress has also been described in human models of hypertension [14]. The importance of ROS in the development and maintenance of hypertension has been recognized for some time [15, 16].

A number of drugs used clinically to reduce the likelihood of developing cardiac disease inflict collateral damage. 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. Cumulative data suggest that these damaging effects are mediated by free radicals and related reactants [17–19].

Cardiac adaptation in response to intrinsic or external stress involves a complex process of chamber remodeling and myocyte molecular modifications [20]. In this context, administration of some exogenous antioxidative compounds has been shown to exert protection against oxidative cardiovascular disorders [21]. The discovery of melatonin as a direct free radical scavenger and as an indirect antioxidant via its stimulatory actions on antioxidative enzymes has greatly increased interest in the potential cardioprotective properties of the indoleamine [3, 22]. The current brief review considers some of cardiac diseases and conditions where free radicals and related

reactants are believed, at least in part, to be causative. In this work, the results of studies on the beneficial effect of melatonin on heart disease are reviewed.

Oxidative stress and melatonin

Oxidative stress is a consequence of the inefficient utilization of molecular oxygen (O_2) by cells [3]. ROS including the superoxide anion radical ($O_2^{\bullet-}$) and hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) are generated as by-products of cellular respiration and other metabolic processes. They damage cellular macromolecules including DNA, proteins, and lipids [23]. Additionally, however, there are also highly devastating agents which are nitrogen based e.g. nitric oxide ($NO^{\bullet}$) and especially the peroxynitrite anion ($ONOO^-$).

Fortunately, organisms are endowed with a series of agents that can either directly detoxify radicals or their associated reactants (free radical scavengers) or they metabolize them to innocuous molecules (antioxidative enzymes) [3]. Melatonin is known to protect against oxidative stress in cells. Antioxidant actions of melatonin are observed at different levels including attenuation of radical formation, which is also referred to as radical avoidance. Although melatonin efficiently interacts with various ROS and reactive nitrogen species as well as with organic radicals, it also upregulates antioxidant enzymes and downregulates pro-oxidant enzymes (Table 1) [24–27]. While the direct free radical scavenging actions of melatonin are receptor independent, the indirect antioxidative functions may well be mediated by receptors, either located in the membranes of cells or within the nucleus [3, 28]. Other important features of a satisfactory antioxidant are its wide distribution within tissues and cells as well as in subcellular compartments, its ability to cross morphophysiologic barriers, and its rapid transport into cells. Melatonin is highly lipid and somewhat aqueous soluble. Thus, melatonin readily enters all cells and subcellular compartments with highest concentrations possibly being present in the nuclei and mitochondria [28].

Table 1. Direct scavenging effects of melatonin on reactive oxygen (ROS) and reactive nitrogen species (RNS) as well as actions on antioxidative/pro-oxidative enzymes

ROS/RNS/enzyme	Effect of melatonin
ROS/RNS	
Hydrogen peroxide	↓
Hydroxyl radical	↓
Singlet oxygen	↓
Nitric oxide	↓
Peroxynitrite anion	↓
Antioxidative enzyme	
Superoxide dismutase	↑
Catalase	↑
Glutathione peroxidase	↑
Glutathione reductase	↑
Glucose-6-phosphate dehydrogenase	↑
Gamma-glutamylcysteine synthase	↑
Pro-oxidative enzymes	
Nitric oxide synthase	↓
Lipo-oxygenase	↓

Melatonin as a free radical scavenger

While melatonin has proven highly effective in lowering molecular damage under conditions of elevated oxidative stress, the actual contribution of this indole in restraining the resulting molecular mutilation that accompanies exaggerated free radical generation remains unknown [1]. Numerous reports have confirmed melatonin's ability to neutralize the highly toxic $^{\bullet}OH$ [2–4, 23, 28–30]. Melatonin reportedly scavenges two $^{\bullet}OH$ and generates the product cyclic-3-hydroxymelatonin (cyclic-3-OHM) [28, 29, 31]. $^{\bullet}OH$ is trapped or added to position 3 of the indole ring of melatonin to form the 3-OHM neutral radical, which undergoes an intramolecular rearrangement to form the cyclic-3-OHM radical. The cycling occurs at position 2 on the indole ring with the nitrogen atom on the side chain. Finally, cyclic-3-OHM radical reacts with a second $^{\bullet}OH$ to form cyclic-3-OHM and a water molecule [31].

The superoxide anion radical ($O_2^{\bullet-}$) is generated when O_2 is reduced by a single electron [29]. The efficacy of melatonin in neutralizing the $O_2^{\bullet-}$ is not well defined. Melatonin was shown to scavenge this reactant in a pure chemical system where a hypoxanthine/xanthine system was used to generate the $O_2^{\bullet-}$. The ability of melatonin to quench the $O_2^{\bullet-}$ is also supported by evidence that melatonin modestly diminished the electron spin resonance signal from spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO-$O_2^{\bullet-}$) adducts. However, the role of melatonin in neutralizing the $O_2^{\bullet-}$ in vivo still requires definition [2].

Recently, it was documented that the immediate precursor of $^{\bullet}OH$, hydrogen peroxide (H_2O_2), is also scavenged by melatonin [32]. H_2O_2 is only a weak oxidizing and reducing agent and has no electric charge. The interaction between the indoleamine and H_2O_2 exhibits two distinguishable phases. In the rapid phase, the interaction reaches equilibrium within 5 s, with a bimolecular rate constant of $2.3 \times 10^6\ m^{-1}s^{-1}$. Thereafter, a second slow reaction phase is characterized by a gradual reduction in H_2O_2 over a several hour period. A mechanism of the oxidation of the indoleamine by H_2O_2 has been proposed on the basis of the main metabolite identified. The reaction leads to the oxidative cleavage of the indole ring to produce a compound identified as AFMK (N^1-acetyl-N^2-formyl-5-methoxykynuramine). Additionally, AFMK is capable of donating two electrons and, therefore, it is also a direct free radical scavenger in its own right.

Singlet oxygen ($^1O_2$) is formed in cells by the photosensitization reaction of substrates such as dyes and biologic pigments. Poeggeler et al. [33] first showed that, upon autooxidation of riboflavin by exposure to bright light, melatonin suppressed the formation of $^1O_2$. In this system, AFMK was identified as a reaction product but no pathway was proposed.

Nitrogen monoxide ($NO^{\bullet}$) is involved in a number of inflammatory process that can cause extensive tissue injury. Additionally, much of the toxicity of $NO^{\bullet}$ may be a consequence of its coupling with $O_2^{\bullet-}$ which results in the formation of the highly reactive $ONOO^-$ [29, 31]. A variety of studies reported that melatonin detoxifies $NO^{\bullet}$ [34–38] and the $ONOO^-$ [2, 39]. Melatonin interacts with $NO^{\bullet}$ only in the presence of molecular oxygen (O_2), a finding

suggesting that melatonin may in fact react with a molecule derived from $NO^{\bullet}$, possibly $ONOO^{-}$. The coupling of two relatively unreactive species ($NO^{\bullet}$ and $O_2^{\bullet-}$), generates the potently oxidizing $ONOO^{-}$. The reactivity of $ONOO^{-}$ must be interpreted in terms of at least three reactive species: an activated form of the acid, $HOONO^{*}$, the ground-state peroxynitrous acid, HOONO and its conjugated base $ONOO^{-}$. Melatonin was shown to react with $ONOO^{-}$ with first-order kinetics; however, the rate constant for the reaction of melatonin with HOONO was considerably higher. Melatonin interacted also with $ONOO^{-}$ but with the formation of different product [29, 31].

Melatonin and antioxidant enzyme activities

The antioxidant defence system may be generally classified into indirect enzymatic antioxidant enzymes and into small molecular weight molecules which directly scavenge free radicals and related reactants. Antioxidant enzymes represent a first line of defence against these toxic reactants by metabolizing them to innocuous by-products [40]. The activation of the major antioxidant enzymes is a consequence of antioxidant enzyme mRNA synthesis and eventually enzyme stimulation. Melatonin's indirect antioxidative actions may well involve specific receptors for the indole. Both membrane (MT1, MT2) and nuclear (Receptor belonging to the retinoid Z receptor/Retinoid related receptor) receptors for melatonin have been identified. MT1 and MT2 receptors are members of the G-protein-linked receptor superfamily [25, 28, 41]. The main antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRd) and glucose-6-phosphate dehydrogenase (G6PD) [42].

One of the most effective intracellular enzymatic antioxidants is SOD. In humans, there are three forms of SOD: cytosolic CuZn-SOD, mitochondrial Mn-SOD and extracellular SOD. Superoxide dismutates $O_2^{\bullet-}$ to H_2O_2, decreasing the amount of $O_2^{\bullet-}$ and thereby lowering the formation of $ONOO^{-}$:

$$2O_2^{\bullet-} + 2H^{+} \xrightarrow{SOD} H_2O_2 + O_2$$

It should be noted that SOD enzymes work in conjunction with H_2O_2-removing enzymes, including CAT and GPx [43–45]. CAT is an enzyme located in the peroxisome. The enzyme very efficiently promotes the conversion of H_2O_2 to H_2O and O_2 [46]:

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

Glutathione peroxidase acts in conjunction with the tripeptide glutathione (GSH), which is present in cells in high (micromolar) concentrations. GPx decomposes peroxides to water while simultaneously oxidizing GSH:

$$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$$

$$GSSG + NADPH + H^{+} \xrightarrow{GRd} 2GSH + NADP^{+}$$

Glutathione peroxidase utilizes GSH as a substrate. Maintaining high intracellular concentrations of GSH seems also to be a function of melatonin as this indole stimulates the activity of its rate-limiting enzyme, γ-glutamylcysteine synthase, in its synthesis. There is one report claiming that melatonin also stimulates G6PD. This would be important in GSH recycling as NADPH is a necessary cofactor for G6PD [2, 29, 45–47].

Besides the antioxidative enzymes mentioned above, the activity of one pro-oxidative enzyme, nitric oxide synthase (NOS), is also altered by melatonin. Melatonin inhibits the activity of NOS and decreases the formation of $NO^{\bullet}$ and the product of its interaction with $O_2^{\bullet-}$, $ONOO^{-}$ [28, 48].

Melatonin activation of MT1/2 receptor, via G inhibitory protein, stimulates the phospholipase C pathway. The consequent increase in Ca^{++} concentration leads to the phosphorylation of protein-kinase C (PKC). PKC activates protein/activation transcriptor factor cAMP responsive element binding protein and activating transcription factor (CREB-ATF). This pathway modulates immediate early gene transcription and consequently gene transcription regulation and antioxidant enzyme levels. An increment of ROS production within cells causes the expression of genes involved in inflammatory processes. The transcriptor factor (NF-κB) is implicated in the transcriptional upregulation of these inflammatory genes. The inactive NF-κB resides in the cytoplasm due to an inhibitory subunit, I-κB. Once the cell is stimulated by an oxidative stress, I-κB is phosphorylated and NF-κB is translocated into the nucleus. PKC activation may also activate NF-κB. In the nucleus, it binds to the κB response element in the enhancer and promoter regions of its target genes. Some of these are located in the promoter regions of the major antioxidant enzymes. Thus, an early cellular response to oxidative stress is an activation of antioxidant enzymes [18, 25, 40].

Melatonin and cardiac ischemia/reperfusion

Ischemia/reperfusion occurs when the blood supply to an organ or region is temporarily interrupted and then restored. The molecular and cellular damages resulting from I/R involves destructive free radicals and related reactants if they are not contested. While interrupting the blood supply to an organ is very serious and leads to tissue death, re-establishing blood flow to the deprived tissue, i.e. reperfusion, is also highly damaging. Melatonin has been widely tested for its ability to attenuate the tissue damage resulting from transient occlusion of the blood supply to organs [3]. Myocardial stunning is the best-established manifestation of reperfusion injury. It is defined as prolonged postischemic mechanical dysfunction that persists after reperfusion of previously ischemic tissue in the absence of irreversible damage, including myocardial necrosis. Myocardial stunning is observed after reperfusion of a globally ischemic myocardium or in the setting of regional ischemia and reperfusion [49]. Reperfusion of the heart after an ischemic period may lead to potentially lethal arrhythmias. In humans, the most common reperfusion arrhythmia is an accelerated idioventricular rhythm. The I/R heart exhibits premature ventricular contractions (PVC) and/or ventricular fibrillation (VF) [1, 49]. Cardiomyocyte death during ischemia is conventionally referred to as oncosis. Development of cardiomyocyte contracture

(contraction band necrosis) seems to be the primary cause for necrotic cardiomyocyte injury during the earliest phase of reperfusion. Thereafter, various additional causes can lead to a further increment in cell death either by necrosis or apoptosis. The myocardium can tolerate brief periods (up to 15 min) of severe and even total myocardial ischemia without the resultant cardiomyocyte death [50].

Endothelial dysfunction is defined as an impaired endothelium-dependent vasodilation, whereas the responses to endothelium-dependent vasoconstrictors are exaggerated. Furthermore, endothelial dysfunction facilitates the expression of a prothrombotic phenotype characterized by platelet and neutrophil activation, which are important mediators of reperfusion injury. It is well known that endothelial dysfunction occurs early during reperfusion of a previously ischemic tissue and that it persists for a long time [49].

Histopathologic changes including intracellular vacuolization, interstitial edema, neutrophil infiltration, hemorrhage, and coagulative necrosis are observed after I/R. Adhesion of neutrophils to endothelium is observed and the first easily recognizable feature of ischemic myocardial injury is the presence of neutrophils in the interstitial tissue. The area of coagulative necrosis is prominent.

Myocardial necrosis is associated with complement activation and free-radical generation, triggering a cytokine cascade and chemokine upregulation [8]. Salie et al. [51] reported that melatonin, via inhibition of ROS generation and intracellular Ca^{2+} accumulation, protects rat ventricular myocytes against I/R-induced morphologic damage. Using a Langendorff rat heart preparation, Tan et al. [52] found that when melatonin was infused throughout the period of occlusion and after reopening of the coronary artery, it highly significantly reduced both the PVC and the VF. The concentration of melatonin in the perfusate was either 1, 10, or 250 μM. Melatonin's effects, in this study, were probably related to its antioxidant action [1].

Additional investigations confirming the beneficial effects of melatonin on the physiology and morphology of the hypoxic/reoxygenated heart have been published. In isolated rat hearts subjected to 30 min ischemia and 30 min perfusion, the infusion of melatonin (100 μM) significantly reduced the duration of ventricular tachycardia and VF and restored ventricular function. Moreover, in this study, $^{\bullet}OH$ generation was measured with melatonin highly significantly attenuating the numbers of this reactant. After the reperfusion period, hearts were collected and the levels of lipid peroxidation were measured in the tissue. Melatonin, again, highly significantly reduced the levels of oxidized lipids in the heart [53]. Simultaneously, Lagneux et al. [54] confirmed that melatonin's free radical scavenging activity reduces both the abnormal cardiac physiology and infarct volume after I/R and restores cardiac function. Additional investigations [7, 55–57] have confirmed the ameliorative effects of melatonin on abnormal function and cardiac tissue destruction resulting from I/R after the administration of pharmacologic doses of melatonin prior to ischemia and/or during reperfusion. Additional studies showed that melatonin also markedly reduced $O_2^{\bullet-}$ production and lowered myloperoxidase activity induced by I/R [1].

A recent report documents the pivotal role of melatonin in limiting myocardial pathophysiology in the I/R rat heart. In this case, an isolated working heart model was used to test melatonin's ability to reduce myocardial damage after transient ischemia followed by reoxygenation [58]. The results of Kacmaz et al. [59] also confirmed that melatonin has a protective effect on I/R-induced oxidative cardiac damage. As the oxidative injury to cellular structures is reduced by melatonin, myeloperoxidase (MPO) activity, which is an index of tissue neutrophil infiltration, is likewise suppressed by melatonin treatment, thus suggesting an anti-inflammatory effect of the indoleamine. Also, the findings of these authors showed that melatonin caused a significant inhibition in MDA levels, thus proving a reduction in lipid peroxidation and cellular injury. The protective effects of melatonin probably occurred, in part, due to the scavenging of any highly reactive $^{\bullet}OH$ and $OONO^{-}$. This group also demonstrated that the drop in GSH levels during I/R was probably due to its consumption during oxidative stress and that melatonin restored GSH levels after I/R.

Sahna et al. [56, 57] were the first to examine whether endogenous, physiologic concentrations of melatonin would change the outcome of such studies. In this case, rats were surgically pinealectomized to reduce endogenous levels of melatonin and, 2 months later, they, along with pineal-intact controls, were used in studies of cardiac I/R injury. When the left coronary artery was occluded for 7 min followed by 7 min of reperfusion, the degree of cardiac arrhythmia was significantly greater in the pinealectomized rats compared with the controls. Even more importantly, the incidence of mortality was 63% in rats lacking their pineal gland compared with only 25% in the pineal-intact rats after I/R induction. These findings suggest that endogenous melatonin levels are protective of the heart during episodes of hypoxia and reoxygenation. Moreover, Sahna et al. [60] assumed that some of melatonin's antioxidant actions probably derive from its stimulatory effect on SOD, GPx, GRd, and G6PD and its inhibitory action on inducible NOS. Experimental evidence has shown that melatonin also promotes the activity of GSH-Rd, thereby helping to maintain high levels of reduced GSH. In addition to being an effective free radical scavenger, melatonin also decreases intracellular calcium concentrations.

Melatonin and atherosclerosis

Cardiovascular diseases (coronary heart disease, stroke, etc.) are often related to atherosclerosis development and remain the major cause of death in most developed countries [61]. Atherosclerosis is a chronic vascular disease in which inflammation and oxidative stress are commonly implicated as major causative factors. The disease process develops and progresses with abnormal cholesterol deposits in the tunica intima of large arteries. Early stages of plaque development involve endothelial activation induced by inflammatory cytokines, oxidized low-density lipoprotein (ox-LDL) and/or changes in endothelial shear stress. This leads initially to the expression of endothelial adhesion molecules and chemokines, followed by the recruitment and activation of circulating monocytes and lymphocytes.

Chronic inflammation appears to play an important role in the initiation and progression of atherosclerosis [62]. It has been clearly demonstrated that hypercholesterolemia secondary to high LDL plasma levels and the subsequent oxidation of these lipoproteins is a major cause of atherosclerosis in humans and different animal models [61]. Human studies have confirmed that oxLDL and oxidized lipid by-products are present in atherosclerotic plaques. LDL particles which accumulate in the plasma infiltrate the intimal space of the arteries and are oxidized by free radicals [63].

Duell et al. [64] tested the capacity of melatonin to inhibit oxidation of LDL in a standardized in vitro system. Although the results of other studies had suggested that melatonin may inhibit LDL oxidation, dose–response data that have compared the capacity of melatonin to inhibit LDL oxidation with other antioxidants is limited. According to Duell et al. [64], melatonin had no antioxidant activity at physiologic concentrations and only moderate antioxidant activity at concentrations that were 4–6 orders of magnitude greater than peak physiologic concentrations.

Several other in vitro studies have investigated the antioxidant effect of melatonin on LDL oxidation. According to Pieri et al. [65] and Kelly and Loo [66], melatonin does inhibit oxidative LDL modification. Furthermore, Seegar et al. [67] demonstrated that, although melatonin itself appears to have little anti-atherogenic activity during LDL oxidation, melatonin's precursors and breakdown products inhibit LDL oxidation, comparable to vitamin E.

Melatonin also has been shown to reduce plasma levels of total cholesterol and of the very low-density lipoprotein (VLDL)-cholesterol plus the LDL cholesterol subfraction in hypercholesterolemic rats. Melatonin may exert the effects by augmenting endogenous cholesterol clearance. One in vitro study demonstrated that melatonin is not incorporated into LDL in sufficient concentrations to prevent lipid peroxidation effectively. However, because of its lipophilic and nonionized nature, melatonin should enter the lipid phase of the LDL particles and prevent lipid peroxidation [68]. Ox-LDL has been reported to inhibit endothelial-dependent relaxation in arteries and decrease expression of NOS in human endothelial cells. Wakatsuki et al. [69] showed that melatonin protected against the oxidized LDL-induced inhibition of NO production in the human umbilical artery. These findings support the hypothesis that melatonin acts as a $^{\bullet}OH$ scavenger in these cells and could potentially protect LDL oxidation.

Vural et al. [70] showed that after 6 wk of treatment, melatonin reduced lipid peroxidation and increased antioxidant enzymes in blood cells. They reported that MDA concentrations decreased in melatonin-treated rats and that melatonin upregulated the antioxidative defense system, and increase the levels of antioxidant enzymes including SOD, GPx, and GRd. Bonnefont-Rousselot et al. [71] evaluated the effect of high concentrations of melatonin on the peroxidation of human LDL. Markers of lipid peroxidation were determined (e.g., decrease in the endogenous antioxidants, alpha-tocopherol and beta-carotene, formation of conjugated dienes and of thiobarbituric acid-reactive substances, TBARS). Melatonin lowered the yields of lipid peroxidation products and delayed the onset of the propagation phase for conjugated dienes and TBARS in a concentration-dependent manner. The effect of melatonin seemed to be greatest at a concentration of 250×10^{-6} M/L.

Atherosclerosis is a chronic inflammatory disorder that results from the interactions between modified lipoproteins and various components of the immune system, including monocyte-derived macrophages, T-lymphocytes and a variety of cytokines secreted by these and other cells in the arterial wall [72]. Anatomic, physiologic, and pharmacologic evidences support the existence of bilateral interactions between endocrine and immune systems. In this context, melatonin plays an important role as a modulator of a large number of cytokines [73]. Melatonin exhibits immunomodulatory properties, which are mediated via membrane and nuclear receptors. Data were reported on activation of T, B, NK cells and monocytes, thymocyte proliferation and release of cytokines (IL-1, IL-2, IL-6, IL-12 and interferon gamma). Anti-inflammatory actions of melatonin are related to the inhibition of PGE_2 effects, and in particular, COX-2 downregulation [26]. Considering the cardiovascular risk of the newly designed COX-2 inhibitors, natural products like melatonin should be considered for use. In a dose-dependent manner, Mayo et al. [74] showed that melatonin inhibited the increase of COX-2 protein expression in a model of lipopolysaccharide-stimulated macrophages. Both COX-2 and iNOS are usually induced following immune stimulation; melatonin also prevented the increase of iNOS. Given that free radicals are involved in the inflammatory process, melatonin is a good candidate as an anti-inflammatory agent, which complements its antioxidant and free radical scavenging features [75].

Melatonin and hypertension

Early data demonstrated that hypertension is the major factor in the development of heart failure. Subsequent epidemiologic studies identified hypertension as a major precursor of heart failure. Hypertension is, of course, not the sole factor contributing to the development of cardiac failure. Multivariate analysis using time-dependent modeling revealed that while myocardial infarction (MI) conferred the greatest risk of developing heart failure, because of its high prevalence, hypertension carried the greatest population-attributable risk. In addition, demographic changes with an aging population are predicted to increase not only the percentage prevalence of hypertension but also, more dramatically, the absolute number of patients diagnosed with the condition [76].

Hypertensive heart disease is characterized histologically by left ventricular (LV) hypertrophy and fibrosis. LV hypertrophy is recognized as a risk factor for cardiovascular morbidity and death, and fibrosis surrounding coronary arteries and myocardial fibers decreases the supply of oxygen and nutrients to the myocardium. These histologic changes, termed 'cardiac remodeling', impair the diastolic function of the LV, often leading to overt heart failure or fatal arrhythmia [77].

Girouard et al. [78] tested the effect of melatonin on the heart of spontaneously hypertensive rats (SHR) with LV hypertrophy. The antihypertensive effect of melatonin was

not accompanied by a reduction of LV relative weight in these animals. Myocyte hypertrophy and myocardial fibrosis, especially reactive fibrosis that expands from the perivascular space to the intermuscular space, are typical features of hypertensive cardiac remodeling [79]. There are only a few studies testing the ability of melatonin to protect the heart from morphologic changes. Microscopic examination of the heart revealed pathologic cardiac changes after pinealectomy [80]. This group noticed myocardial fibrosis and degeneration of the valves in both pinealectomized rats and pinealectomized rats treated with melatonin. However, they reported increased levels of MDA and cholesterol and reduced levels of GSH in the heart of pinealectomized groups, which were restored by melatonin treatment. Thus, the administration of melatonin to pinealectomized rats had beneficial effects as indicated by lower levels of cholesterol and MDA. However, melatonin treatment reportedly did not have similar effect on the morphology of the cardiovascular system. Failure to restore normal cardiac morphology was probably due to melatonin's relatively short-term application, as it was given for only 3 days in rats that had been pinealectomized for 2 months.

Additional studies demonstrated an increase in plasma MDA and in the imbalance between oxidized and reduced glutathione (GSSG:GSH), an index of oxidized thiols, in SHRs. In that study, chronic treatment with melatonin caused normalization of plasma MDA and of GSSG:GSH in SHRs. These results thus suggest that the doses of melatonin (30 mg/kg) used were sufficient to decrease the production of free radicals, resulting in a reduced cell damage including membrane lipid peroxidation and the oxidation of thiols in SHRs [81].

An impairment of endothelial function has been detected in conduit and resistance arteries both in animal and human hypertension. Structural abnormalities include cardiac hypertrophy and hypertrophy of the tunica media in conduit and resistance arteries. These commonly accompany chronic hypertension and play an important role in the increase of vascular resistance, and therefore in the maintenance of high blood pressure (BP, [82]).

The administration of melatonin has been reported to reduce BP as a consequence of various mechanisms including a direct hypothalamic effect, a lowering of catecholamine levels, relaxation of the smooth muscle wall and, most importantly, as a result of its antioxidant properties [83]. There are several reports indicating that melatonin may have a hypotensive effect. Nava et al. [16] demonstrated that, in SHR, melatonin treatment for 6 wk reduced oxidative stress. SHR exhibit a progressive rise in systolic BP (SBP) over the same 6 wk period. SBP was gradually reduced following treatment of SHR with melatonin. Both intracellular $O_2^{\bullet -}$ production and MDA levels were drastically reduced in SHR given melatonin. The melatonin-treated animals exhibited a significant reduction in the expression of NF-κB, an effect that may indirectly contribute to melatonin's role as an antioxidant.

In a similar study, Simko and Paulis [84] demonstrated that, in SHR, BP decreased after 6 wk of melatonin treatment and, moreover, they showed also a reduction in interstitial renal tissue inflammation, decreased oxidative stress, and attenuation of expression of renal NF-κB. Long-term treatment with melatonin increased the antioxidant reserve by normalizing the depressed GPx activity in SHR. The use of chronic melatonin treatment, moreover, also was found to normalize plasma MDA and the GSSG:GSH. These results suggest that the dose of melatonin (30 mg/kg) used in the present study is sufficient to increase the antioxidant reserve resulting in a reduction in cell damage including membrane peroxidation and oxidation of thiols, which are typically observed in SHR [78].

Scheer et al. [85] investigated the effect of melatonin intake in patients with essential hypertension. They found that the indoleamine reduced BP while the subjects slept. This reduction due to melatonin is important considering humans sleep approximately one-third of their lives and because nighttime BP seems to be a better predictor of cardiovascular risk than is daytime BP.

It is known that NO plays a key role in the maintenance of vascular tone affecting BP. A relative NO deficiency has been documented in different forms of hypertension. Pechanova et al. [86] demonstrated that melatonin reduces BP significantly and that the treatment enhanced NO synthase activity, reduced oxidative stress, and decreased NF-κB. A possible explanation for the findings is a direct effect of melatonin on endothelial intracellular Ca^{++} concentrations, which would enhance NOS activity. Indeed, elevated intracellular Ca^{++} levels in endothelial cells were observed after melatonin treatment.

Melatonin and clinically used drugs

In addition to their beneficial effects, a number of clinically useful drugs inflict collateral damage when they are administered. The side effects often are mediated by free radicals and related reactants. Doxorubicin, cisplatin, epirubicin, bleomycin, and cytarabine are agents used to treat a variety of tumors. The molecular mechanisms which account for the tissue damage resulting from the use of these drugs have been partially identified and the generation of free radicals, GSH depletion, elevated levels of lipid peroxidation products, increased DNA fragmentation and the inhibition of DNA synthesis and repair are considered significant aspects of the resulting tissues damage.

Cyclosporine A (CsA), an immunosuppressant agent, has significant side effects that involve kidney, liver, and heart. Iron and erythropoietin, used for the treatment of anemia, also have side effects which likely involve free radical damage. For inhibition of epileptic activity, psychoses and agitation, phenobarbital, carbamazepine, and haloperidol are used. They also provoke neurologic and psychomotor deterioration, DNA damage, and an increase of the oxidative stress. Cyclophosphamide, an alkylating agent, induces DNA damage and L-cysteine, used for the treatment of erythropoietic protoporphyria, causes an increase of lipid peroxidation products [17].

Like CsA, doxorubicin causes cardiac damage [18, 87]. Doxorubicin is commonly used for the treatment of cancer. There are serious toxic effects on the cardiovascular system, which limit the application of the drug. Pharmacologic concentrations of melatonin have been shown to limit doxorubicin-induced cardiac injury in the rat heart. The

histologic examination of the heart of doxorubicin-treated rats showed severe morphologic damage. Inflammatory cell infiltration and myocardial fibrosis were apparent. Moreover, examination revealed destruction of myofibrils, disorganization of sarcomeres, mitochondrial degeneration, and formation of giant mitochondria and lipid accumulation. An ultrastructural analysis of the heart of doxorubicin-treated rats showed the presence of cellular edema, mitochondrial deformation, decrease glycogen stores, and disordered myofibrillary structures. When melatonin was added to the doxorubicin treatment, all structural changes were reduced and normal cellular morphology was observed [57, 87, 88]. Additionally, doxorubicin generates large numbers of oxygen-based radicals, which damage cardiomyocytes and decreases the levels of GSH which markedly lowers the efficiency of the endogenous antioxidative system of the heart. Reduced levels of MDA and augmented levels of GSH were found after melatonin administration. Most likely melatonin increased GSH levels via stimulation of the rate-limiting enzyme, γ-glutamylcysteine synthase. GSH is an efficient scavenger of free oxygen radicals and a substrate indispensable for GPx function. SOD activity was significantly elevated, which is important for the scavenging high numbers of the $O_2^{\bullet-}$. The findings suggest that SOD activity in cardiomyocytes subjected to doxorubicin possibly represents an important facet of the inactivation of $O_2^{\bullet-}$. The most interesting result involved was CAT. This enzyme decomposes H_2O_2. In doxorubicin-treated rats, CAT activity was higher than in control animals. It was further increased in rats that received both doxorubicin plus melatonin. These findings indicate that there is increased protection by antioxidative enzyme as a result of melatonin administration to doxorubicin-treated rats [89].

Cyclosporine A is currently the most widely used immunosuppressive drug for preventing graft rejection and autoimmune diseases. The clinical use of CsA is limited because of its side effects at the cardiac level [90]. CsA induces degenerative myocardial changes involving size, shape, and organization of the cardiomyocytes; these effects are triggered by enhanced oxidative stress [91]. The striated muscle fibers in the heart are disorganized and the networks are reduced. The connective tissue is clearly increased and the collagen volume fraction is significantly greater compared with the heart of the control rats [92]. Studies to elucidate the mechanism(s) of these adverse effects have implicated free radical production, lipid peroxidation, and induction of the cytochrome P450 system and increased synthesis of vasoconstrictor eicosanoids [93]. Clinical and experimental data suggest that ROS such as $O_2^{\bullet-}$ and H_2O_2, which cause minimal damage at physiologic levels, at high concentrations can cause cellular damage and may play a key role in drug-induced toxicity [94].

Rezzani et al. [18] investigated the effects of melatonin on CsA-induced cardiotoxicity. Histologic changes in the heart of CsA-treated animals included a massive increase in the number of infiltrated cells and disorganization of myocardial fibers with interstitial fibrosis, all of which disappeared after melatonin administration. TBARS also were higher in CsA-treated animals while melatonin treatment significantly reduced the levels of lipid peroxidation. CsA treatment was accompanied by significant reductions in GSH levels and in the activities of both CAT and SOD. These reductions were restored by melatonin administration. The authors also tested whether the protective effects of melatonin against CsA toxicity in the heart were mediated by the direct scavenging actions of melatonin or because of its indirect stimulatory actions on antioxidative enzymes. The administration of the high affinity melatonin receptor antagonist, luzindole, showed that melatonin's beneficial effects were completely abolished. Thus, melatonin's protective actions against CsA were probably as a result of promotion of antioxidative enzyme activities rather than due to its free radical scavenging activity of the indoleamine.

Conclusions

The collective results show that several cardiac conditions are a consequence of free radical damage and processes involving an inflammatory response. The beneficial effects of melatonin administration against these conditions are due to its direct free radical scavenger activity, its indirect antioxidant properties and its anti-inflammatory effects. When exogenously administered, melatonin is quickly distributed throughout the organism. It crosses all morphophysiologic barriers and enters cardiac cells with ease. Highest intracellular concentrations of melatonin seem to be in the mitochondria. This is especially important as mitochondria are a major site of free radical generation and oxidative stress. Finally, melatonin's virtual absence of toxicity makes possible its long-term use [29]. Collectively, these protective actions of melatonin may also have potential clinical applicability for individuals with cardiovascular disease including those treated with angioplasty [95].

Recent studies have documented that at least some of melatonin's antioxidant effects are in fact due to its metabolites which are generated when melatonin interacts with free radicals [96–100]. Furthermore, melatonin's ability to inhibit the pro-oxidative enzyme, NOS, is mediated by a tertiary metabolite of melatonin, AFMK [101]. The ability of the parent molecule melatonin as well as its metabolites to function in radical detoxification greatly increases its ability to limit oxidative abuse at many levels within cells.

References

1. Reiter RJ, Tan DX. Melatonin: a novel protective agent against oxidative injury of the ischemic/reperfused heart. Cardiovasc Res 2003; **58**:10–19.
2. Reiter RJ, Tan DX, Mayo JC, Sainz RM, Leon J, Czarnocki Z. Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. Acta Biochim Pol 2003; **50**:1129–1146.
3. Reiter RJ, Tan DX, Gitto E et al. Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. Pol J Pharmacol 2004; **56**:159–170.
4. Anisimov VN, Popovich IG, Zabezhinski MA, Anisimov SV, Vesnushkin GM, Vinogradova IA. Melatonin as

antioxidant, geroprotector and anticarcinogen. Biochim Biophys Acta 2006; **1757**:573–589.
5. Flora SJ. Role of free radicals and antioxidants in health and disease. Cell Mol Biol (Noisy-le-grand) 2007; **53**:1–2.
6. Kloner RA, Przyklenk K, Whittaker P. Deleterious effects of oxygen radicals in ischemia/reperfusion. Resolved and unresolved issues. Circulation 1989; **80**:1115–1127.
7. Szarszoi O, Asemu G, Vanecek J, Ost'adal B, Kolar F. Effects of melatonin on ischemia and reperfusion injury of the rat heart. Cardiovasc Drugs Ther 2001; **15**:251–257.
8. Esrefoglu M, Gul M, Parlakpinar H, Acet A. Effects of melatonin and caffeic acid phenethyl ester on testicular injury induced by myocardial ischemia/reperfusion in rats. Fundam Clin Pharmacol 2005; **19**:365–372.
9. Baljinnyam E, Hasebe N, Morihira M et al. Oral pretreatment with ebselen enhances heat shock protein 72 expression and reduces myocardial infarct size. Hypertens Res 2006; **29**:905–913.
10. Petrosillo G, Di Venosa N, Pistolese M et al. Protective effect of melatonin against mitochondrial dysfunction associated with cardiac ischemia–reperfusion: role of cardiolipin. FASEB J 2006; **20**:269–276.
11. Papaharalambus CA, Griendling KK. Basic mechanisms of oxidative stress and reactive oxygen species in cardiovascular injury. Trends Cardiovasc Med 2007; **17**:48–54.
12. Surekha RH, Srikanth BB, Jharna P, Ramachandra RV, Dayasagar RV, Jyothy A. Oxidative stress and total antioxidant status in myocardial infarction. Singapore Med J 2007; **48**:137–142.
13. Li JJ, Fang CH, Hui RT. Is hypertension an inflammatory disease? Med Hypotheses 2005; **64**:236–240.
14. Alvarez Y, Perez-Giron JV, Hernanz R et al. Losartan reduces the increased participation of cyclooxygenase-2-derived products in vascular responses of hypertensive rats. J Pharmacol Exp Ther 2007; **321**:381–388.
15. Berry C, Brosnan MJ, Fennell J, Hamilton CA, Dominiczak AF. Oxidative stress and vascular damage in hypertension. Curr Opin Nephrol Hypertens 2001; **10**:247–255.
16. Nava M, Quiroz Y, Vaziri N, Rodriguez-Iturbe B. Melatonin reduces renal interstitial inflammation and improves hypertension in spontaneously hypertensive rats. Am J Physiol Renal Physiol 2003; **284**:F447–F454.
17. Reiter RJ, Tan DX, Sainz RM, Mayo JC, Lopez-Burillo S. Melatonin: reducing the toxicity and increasing the efficacy of drugs. J Pharm Pharmacol 2002; **54**:1299–1321.
18. Rezzani R, Rodella LF, Bonomini F, Tengattini S, Bianchi R, Reiter RJ. Beneficial effects of melatonin in protecting against cyclosporine A-induced cardiotoxicity are receptor mediated. J Pineal Res 2006; **41**:288–295.
19. Guven A, Yavuz O, Cam M, Ercan F, Bukan N, Comunoglu C. Melatonin protects against epirubicin-induced cardiotoxicity. Acta Histochem 2007; **109**:52–60.
20. Takimoto E, Kass DA. Role of oxidative stress in cardiac hypertrophy and remodeling. Hypertension 2007; **49**:241–248.
21. Cao Z, Zhu H, Zhang L, Zhao X, Zweier JL, Li Y. Antioxidants and phase 2 enzymes in cardiomyocytes: chemical inducibility and chemoprotection against oxidant and simulated ischemia–reperfusion injury. Exp Biol Med (Maywood) 2006; **231**:1353–1364.
22. Lochner A, Genade S, Davids A, Ytrehus K, Moolman JA. Short- and long-term effects of melatonin on myocardial post-ischemic recovery. J Pineal Res 2006; **40**:56–63.
23. Kozina LS, Arutjunyan AV, Khavinson VKh. Antioxidant properties of geroprotective peptides of the pineal gland. Arch Gerontol Geriatr 2007; **1**:213–216.
24. Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. Endocrine 2005; **27**:119–130.
25. Tomas-Zapico C, Coto-Montes A. A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. J Pineal Res 2005; **39**:99–104.
26. Hardeland R, Pandi-Perumal SR, Cardinali DP. Melatonin. Int J Biochem Cell Biol. 2006; **38**:313–316.
27. Suzen S, Bozkaya P, Coban T, Nebiogu D. Investigation of the in vitro antioxidant behaviour of some 2-phenylindole derivatives: discussion on possible antioxidant mechanisms and comparison with melatonin. J Enzyme Inhib Med Chem 2006; **21**:405–411.
28. 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.
29. Reiter RJ, Tan DX, Burkhardt S. Reactive oxygen and nitrogen species and cellular and organismal decline: amelioration with melatonin. Mech Ageing Dev 2002; **123**:1007–1019.
30. Tan DX, Reiter RJ, Manchester LC et al. Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. Curr Top Med Chem 2002; **2**:181–197.
31. Allegra M, Reiter RJ, Tan DX, Gentile C, Tesoriere L, Livrea MA. The chemistry of melatonin's interaction with reactive species. J Pineal Res 2003; **34**:1–10.
32. Tan DX, Manchester LC, Reiter RJ et al. Melatonin directly scavenges hydrogen peroxide: a potentially new metabolic pathway of melatonin biotransformation. Free Radic Biol Med 2000; **29**:1177–1185.
33. Poeggeler B, Saarela S, Reiter RJ et al. Melatonin – a highly potent endogenous radical scavenger and electron donor: new aspects of the oxidation chemistry of this indole accessed in vitro. Ann N Y Acad Sci 1994; **738**:419–420.
34. Mahal HS, Sharma HS, Mukherjee T. Antioxidant properties of melatonin: a pulse radiolysis study. Free Radic Biol Med 1999; **26**:557–565.
35. Noda Y, Mori A, Liburdy R, Packer L. Melatonin and its precursors scavenge nitric oxide. J Pineal Res 1999; **27**:159–163.
36. Blanchard B, Pompon D, Ducrocq C. Nitrosation of melatonin by nitric oxide and peroxynitrite. J Pineal Res 2000; **29**:184–192.
37. Turjanski A, Chaia ZD, Doctorovich F, Estrin D, Rosenstein R, Piro OE. *N*-nitrosomelatonin. Acta Crystallogr C 2000; **56**:682–683.
38. Turjanski AG, Leonik F, Rosenstein RE et al. Scavenging of NO by melatonin. J Am Chem Soc 2000; **122**:10468–10469.
39. Srinivasan V. Melatonin oxidative stress and neurodegenerative diseases. Indian J Exp Biol 2002; **40**:668–679.
40. Rodriguez C, Mayo JC, Sainz RM et al. Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res 2004; **36**:1–9.
41. Barrett P, Conway S, Morgan PJ. Digging deep – structure–function relationships in the melatonin receptor family. J Pineal Res 2003; **35**:221–230.
42. Escames G, Acuna-Castroviejo D, Lopez LC et al. Pharmacological utility of melatonin in the treatment of septic

shock: experimental and clinical evidence. J Pharm Pharmacol 2006; **58**:1153–1165.
43. Kato M, Minakami H, Kuroiwa M et al. Superoxide radical generation and Mn- and Cu–Zn superoxide dismutases activities in human leukemic cells. Hematol Oncol 2003; **21**:11–16.
44. Kruidenier L, Kuiper I, Van Duijn W et al. Imbalanced secondary mucosal antioxidant response in inflammatory bowel disease. J Pathol 2003; **201**:17–27.
45. Rahman I, Biswas SK, Kode A. Oxidant and antioxidant balance in the airways and airway diseases. Eur J Pharmacol 2006; **533**:222–239.
46. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006; **160**:1–40.
47. Carampin P, Rosan S, Dalzoppo D, Zagotto G, Zatta P. Some biochemical properties of melatonin and the characterization of a relevant metabolite arising from its interaction with H_2O_2. J Pineal Res 2003; **34**:134–142.
48. Aydogan S, Yerer MB, Goktas A. Melatonin and nitric oxide. J Endocrinol Invest 2006; **29**:281–287.
49. Moens AL, Claeys MJ, Timmermans JP, Vrints CJ. Myocardial ischemia/reperfusion-injury, a clinical view on a complex pathophysiological process. Int J Cardiol 2005; **100**:179–190.
50. Piper HM, Meuter K, Schafer C. Cellular mechanisms of ischemia–reperfusion injury. Ann Thorac Surg 2003; **75**:S644–S648.
51. Salie R, Harper I, Cillie C et al. Melatonin protects against ischaemic–reperfusion myocardial damage. J Mol Cell Cardiol 2001; **33**:343–357.
52. Tan DX, Manchester LC, Reiter RJ, Qi W, Kim SJ, El-Sokkary GH. Ischemia/reperfusion-induced arrhythmias in the isolated rat heart: prevention by melatonin. J Pineal Res 1998; **25**:184–191.
53. Kaneko S, Okumura K, Numaguchi Y et al. Melatonin scavenges hydroxyl radical and protects isolated rat hearts from ischemic reperfusion injury. Life Sci 2000; **67**:101–112.
54. Lagneux C, Joyeux M, Demenge P, Ribuot C, Godin-Ribuot D. Protective effects of melatonin against ischemia–reperfusion injury in the isolated rat heart. Life Sci 2000; **66**:503–509.
55. Lee YM, Chen HR, Hsiao G, Sheu JR, Wang JJ, Yen MH. Protective effects of melatonin on myocardial ischemia/reperfusion injury in vivo. J Pineal Res 2002; **33**:72–80.
56. Sahna E, Olmez E, Acet A. Effects of physiological and pharmacological concentrations of melatonin on ischemia–reperfusion arrhythmias in rats: can the incidence of sudden cardiac death be reduced? J Pineal Res 2002; **32**:194–198.
57. Sahna E, Parlakpinar H, Ozer MK, Ozturk F, Ozugurlu F, Acet A. Melatonin protects against myocardial doxorubicin toxicity in rats: role of physiological concentrations. J Pineal Res 2003; **35**:257–261.
58. Dobsak P, Siegelova J, Eicher JC et al. Melatonin protects against ischemia–reperfusion injury and inhibits apoptosis in isolated working rat heart. Pathophysiology 2003; **9**:179–187.
59. Kacmaz A, User EY, Sehirli AO, Tilki M, Ozkan S, Sener G. Protective effect of melatonin against ischemia/reperfusion-induced oxidative remote organ injury in the rat. Surg Today 2005; **35**:744–750.
60. Sahna E, Parlakpinar H, Turkoz Y, Acet A. Protective effects of melatonin on myocardial ischemia/reperfusion induced infarct size and oxidative changes. Physiol Res 2005; **54**:491–495.
61. Tailleux A, Gozzo A, Torpier G et al. Increased susceptibility of low-density lipoprotein to ex vivo oxidation in mice transgenic for human apolipoprotein B treated with 1 melatonin-related compound is not associated with atherosclerosis progression. J Cardiovasc Pharmacol 2005; **46**:241–249.
62. Rodella FL, Bonomini F, Rezzani R et al. Atherosclerosis and the protective role played by different proteins in apolipoprotein E-deficient mice. Acta Histochem 2007; **109**:45–51.
63. Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez M, Ferrer-Hita J, Vargas M, Reiter RJ. Elevated levels of oxidized low-density lipoprotein and impaired nocturnal synthesis of melatonin in patients with myocardial infarction. Atherosclerosis 2005; **180**:101–105.
64. Duell PB, Wheaton DL, Shultz A, Nguyen H. Inhibition of LDL oxidation by melatonin requires supraphysiologic concentrations. Clin Chem 1998; **44**:1931–1936.
65. Pieri C, Marra M, Gáspár R, Damjanovich S. Melatonin protects LDL from oxidation but does not prevent the apolipoprotein derivatization. Biochem Biophys Res Commun 1996; **222**:256–260.
66. Kelly MR, Loo G. Melatonin inhibits oxidative modification of human low-density lipoprotein. J Pineal Res 1997; **22**:203–209.
67. Seegar H, Mueck AO, Lippert TH. Effect of melatonin and metabolites on copper-mediated oxidation of flow density lipoprotein. Br J Clin Pharmacol 1997; **44**:283–284.
68. Wakatsuki A, Okatani Y, Ikenoue N, Izumiya C, Kaneda C. Melatonin inhibits oxidative modification of low-density lipoprotein particles in normolipidemic postmenopausal women. J Pineal Res 2000; **28**:136–142.
69. Wakatsuki A, Okatani Y, Ikenoue N, Shinohara K, Watanabe K, Fukaya T. Melatonin protects against oxidized low-density lipoprotein-induced inhibition of nitric oxide production in human umbilical artery. J Pineal Res 2001; **31**:281–288.
70. Vural H, Sabuncu T, Arslan SO, Aksoy N. Melatonin inhibits lipid peroxidation and stimulates the antioxidant status of diabetic rats. J Pineal Res 2001; **31**:193–198.
71. Bonnefont-Rousselot D, Guilloz V, Lepage S et al. Protection of endogenous beta-carotene in LDL oxidized by oxygen free radicals in the presence of supraphysiological concentrations of melatonin. Redox Rep 2003; **8**:95–104.
72. Wilund KR. Is the anti-inflammatory effect of regular exercise responsible for reduced cardiovascular disease? Clin Sci (Lond) 2007; **112**:543–555.
73. Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ. A review of the multiple actions of melatonin on the immune system. Endocrine 2005; **27**:189–200.
74. Mayo JC, Sainz RM, Tan DX et al. Anti-inflammatory actions of melatonin and its metabolites, *N*1-acetyl-*N*2-formyl-5-methoxykynuramine (AFMK) and *N*1-acetyl-5-methoxykynuramine (AMK), in macrophages. J Neuroimmunol 2005; **165**:139–149.
75. Cuzzocrea S, Thiemermann C, Salvemini D. Potential therapeutic effect of antioxidant therapy in shock and inflammation. Curr Med Chem 2004; **11**:1147–1162.
76. Meredith PA, Ostergren J. From hypertension to heart failure – are there better primary prevention strategies? J Renin Angiotensin Aldosterone Syst 2006; **7**:64–73.

77. Masuyama H, Tsuruda T, Kato J et al. Soluble guanylate cyclase stimulation on cardiovascular remodeling in angiotensin II-induced hypertensive rats. Hypertension 2006; **48**:972–978.
78. Girouard H, Denault C, Chulak C, De Champlain J. Treatment by *n*-acetylcysteine and melatonin increases cardiac baroreflex and improves antioxidant reserve. Am J Hypertens 2004; **17**:947–954.
79. Kai H, Mori T, Tokuda K et al. Pressure overload-induced transient oxidative stress mediates perivascular inflammation and cardiac fibrosis through angiotensin II. Hypertens Res 2006; **29**:711–718.
80. Mizrak B, Parlakpinar H, Acet A, Turkoz Y. Effects of pinealectomy and exogenous melatonin on rat hearts. Acta Histochem 2004; **106**:29–36.
81. Girouard H, Chulak C, Le Jossec M, Lamontagne D, De Champlain J. Chronic antioxidant treatment improves sympathetic functions and beta-adrenergic pathway in the spontaneously hypertensive rats. J Hypertens 2003; **21**:179–188.
82. Torok J, Koprdova R, Cebova M, Kunes J, Kristek F. Functional and structural pattern of arterial responses in hereditary hypertriglyceridemic and spontaneously hypertensive rats in early stage of experimental hypertension. Physiol Res 2006; **55**:S65–S71.
83. Sewerynek E. Melatonin and the cardiovascular system. Neuro Endocrinol Lett 2002; **23**:79–83.
84. Simko F, Paulis L. Melatonin as a potential antihypertensive treatment. J Pineal Res 2007; **42**:319–322.
85. Scheer FA, Van Montfrans GA, Van Someren EJ, Mairuhu G, Buijs RM. Daily nighttime melatonin reduces blood pressure in male patients with essential hypertension. Hypertension 2004; **43**:192–197.
86. Pechanova O, Zicha J, Paulis L et al. The effect of *N*-acetylcysteine and melatonin in adult spontaneously hypertensive rats with established hypertension. Eur J Pharmacol 2007; **561**:129–136.
87. Oz E, Erbas D, Surucu HS, Duzgun E. Prevention of doxorubicin-induced cardiotoxicity by melatonin. Mol Cell Biochem 2006; **282**:31–37.
88. Balli E, Mete UO, Tuli A, Tap O, Kaya M. Effect of melatonin on the cardiotoxicity of doxorubicin. Histol Histopathol 2004; **19**:1101–1108.
89. Dziegiel P, Murawska-Cialowicz E, Jethon Z et al. Melatonin stimulates the activity of protective antioxidative enzymes in myocardial cells of rats in the course of doxorubicin intoxication. J Pineal Res 2003; **35**:183–187.
90. Rezzani R, Rodella L, Dessy C, Daneau G, Bianchi R, Feron O. Changes in Hsp90 expression determine the effects of cyclosporine A on the NO pathway in rat myocardium. FEBS Lett 2003; **552**:125–129.
91. Rezzani R. Exploring cyclosporine A-side effects and the protective role-played by antioxidants: the morphological and immunohistochemical studies. Histol Histopathol 2006; **21**:301–316.
92. Bianchi R, Rodella L, Rezzani R. Cyclosporine A up-regulates expression of matrix metalloproteinase 2 and vascular endothelial growth factor in rat heart. Int Immunopharmacol 2003; **3**:427–433.
93. Rezzani R, Giugno L, Buoli B, Bonomini F, Bianchi R. The protective effect of caffeic acid phenethyl ester against cyclosporine A-induced cardiotoxicity in rats. Toxicology 2005; **212**:155–164.
94. Florio S, Ciarcia R, Crispino L et al. Hydrocortisone has a protective effect on CyclosporinA-induced cardiotoxicity. J Cell Physiol 2003; **195**:21–26.
95. Dominguez-Rodriquez A, Abreu-Gonzalez P, Garcia-Gonzalez MJ, Kaski JC, Reiter RJ, Jiminez-Sosa A. A unicenter randomized, double-blind, parallel-group, placebo-controlled study of Melatonin as an Adjunct in patients with acute myocaRdial Infarction undergoing primary Angioplasty. The Melatonin Adjunct in the acute myocaRdial Infarction treated with Angioplasty (MARIA) trail: study design and rationale. Contemp Clin Trials 2007; **28**:532–539.
96. Onuki J, Almeida EA, Medeiros MHG, Mascio PD. Inhibition of 5-aminolevulenic acid-induced DNA damage by melatonin, N^1-acetyl-N^2-formyl-5-methoxykynuramine, quercetin or resveratrol. J Pineal Res 2005; **38**:107–115.
97. Rosen J, Than NN, Koch D, Poeggeler B, Laatsch H, Hardeland R. Interactions of melatonin in and its metabolites with the ABTS cation radical: extension of the radical scavenger cascade and formation of a novel class of oxidation products, C2-substituted 3-indolinones. J Pineal Res 2006; **41**:374–381.
98. Zavodnik IB, Domanski AV, Lapshina EA, Bryszewska M, Reiter RJ. Melatonin directly scavenges free radicals generated in red blood cells and a cell-free system: chemiluminescence measurements and theoretical calculations. Life Sci 2006; **79**:391–400.
99. Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res 2007; **42**:28–42.
100. Manda K, Veno M, Anzai K. AFMK, a melatonin metabolite, attenuates X-ray-induced oxidative damage to DNA, proteins and lipids in mice. J Pineal Res 2007; **42**:386–393.
101. Leon J, Escames G, Rodriguez MI et al. Inhibition of neuronal nitric oxide synthase activity by *N*1-acetyl-5-methoxykynuramine, a brain metabolite of melatonin. J Neurochem 2006; **98**:2023–2033.