



The Antioxidant Effect of Melatonin on Myocardial Ischemia Reperfusion Injury in Experimental Studies

Hakan Parlakpınar¹, Mehmet Hamdi Orum²

¹ Inonu University, Faculty of Medicine, Department of Pharmacology, Malatya, Turkey

² Inonu University, Faculty of Medicine, Under-Graduated Student (Class 6), Malatya, Turkey

Abstract

A lot of mechanisms play major role in the myocardial ischemia reperfusion injury (MI/R), especially reactive oxygen species (ROS) which they formed rapidly when molecular oxygen enters into the cell. Melatonin, major hormone of the pineal gland, has very strong antioxidant and free radical scavenger effects. Besides, melatonin plays an important role in the regulation of various body functions including circadian sleep rhythms, blood pressure, oncogenesis, retinal function, and immunity. It can be possible to say that oxidative stress occurs during MI/R and melatonin administration exerts an infarct size limiting effect, antiapoptotic, antiarrhythmic effects and enhances antioxidant activity of the heart.

Key words: Myocardial ischemia-reperfusion, free radicals, no-reflow, necrosis, antioxidants

(Rec.Date: May 28, 2014

Accept Date: Jun 02, 2014)

Corresponding Author: Hakan Parlakpınar, Inonu University, Faculty of Medicine, Department of Pharmacology, Malatya, Turkey

E-mail: hparlakpınar@inonu.edu.tr **Phone:** +90 341 06 60-1227, **Fax:** +90422 3410036

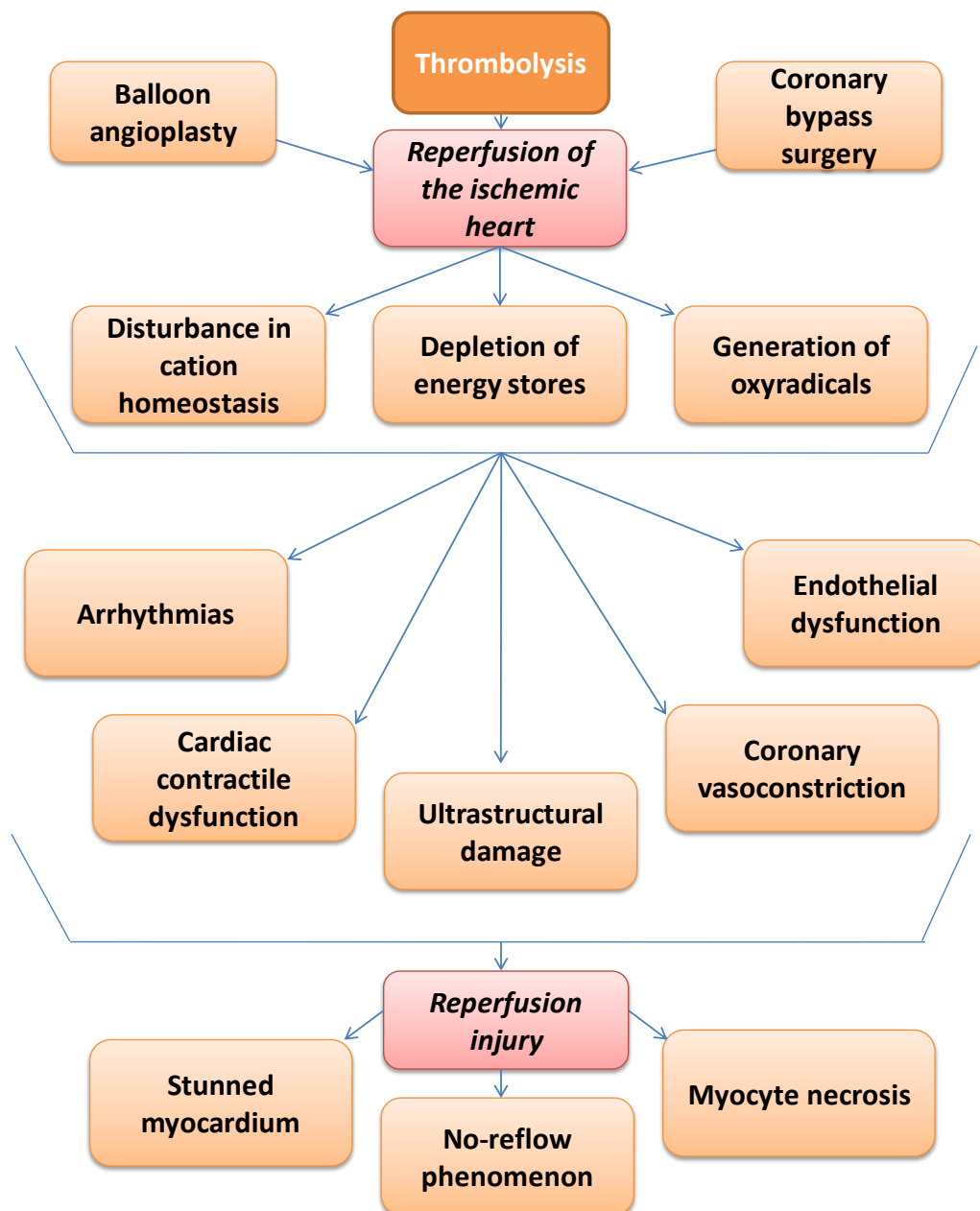
Introduction

Ischemic heart diseases occur when blood supply to the myocardial tissue is insufficient as a result of thrombus, atherosclerotic plaques, vasoconstriction or inflammation. Those were sourced most often from coronary atherosclerosis. In myocardial ischemia, cellular energy stores decrease, metabolic wastes increase and these circumstances lead to the cell death by necrotic and apoptotic pathways. This situation results in severe problems such as fatal arrhythmias and myocardial infarctus (MI) (Fig 1).

Reflow of blood to the ischemic area must be provided in a short time for either the regeneration of cells or clear the toxic metabolites. However, reperfusion of ischemic myocardium leads to more severe damage paradoxically, compared with ischemic injury [1]. A lot of mechanisms play major role in the reperfusion injury, especially reactive oxygen species (ROS) which they formed rapidly when molecular oxygen enters into the cell. Membrane lipids, proteins, nucleic acids and deoxyribonucleic acid molecules are the most sensitive cellular conformations to ROS formation [2].

Myocardial Ischemia Reperfusion (MI/R) Injury

Metabolic and structural changes eventually occur at ischemic period. With the cessation of blood flow to the tissue, cellular oxydative phosphorylation and high energy phosphate synthesis such as ATP (adenosine 5'-triphosphate) and phosphocreatinine absolutely decrease. After energy stores are discharged, Na⁺-K⁺ ATP-ase pump in the cell membrane is inhibited. Eventually, intracellular Na⁺ and Ca²⁺ ion concentrations become to increase. It is well established that the increases in Ca²⁺ ion concentration in the cell exert cytotoxic effects to the viable cell. During this period, with the alteration of ion concentration, the production of proinflammatory cytokines and leukocyte adhesion molecules increase whereas the formation of antioxidant enzymes decreases. This situation triggers the exposed cell vulnerable to damage during reperfusion [3].

Fig 1. Algorithm of the myocardial ischemia reperfusion injury

The reperfusion of ischemic myocardium leads to formation of ROS. These metabolites (superoxide anion (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2)) are observed as one of the important reasons of reperfusion injury. ROS has an unpaired electron attacks all biomolecules in the cell due to its high reactivity [4,5]. This event which is a cascade of ongoing may last until scavenging of radicals and removing of peroxides from environment. The antioxidants such as superoxide dismutase (SOD), catalase, glutathione

peroxidase (GSH-Px), glutathione and vitamin E create a line of defense against oxidative stress. The deterioration of the balance between formation rate of oxidant molecules and antioxidant defenses leads to oxidative stress [6,7].

Melatonin

The presence of the pineal gland has been known since ancient times. The famous philosopher Descartes (1596-1650) believes that the pineal gland is "the village inhabited by the soul". Although known for a long time, the main substance secreted by the pineal gland, however at the end of the 1950s, it has been shown to be melatonin. There is evidence that there might be the role of melatonin in the regulation of many biological events such as circadian rhythm, sleep, reproduction, mental status, tumor development and aging. The most important factor determining the speed of secretion of melatonin is regarding with the environment whether bright or dark. In general, the light inhibits melatonin production whereas it is increased in dark phase [8-10]. In the synthesis of melatonin is indol amino acid, "tryptophan" is essential and it is taken from plasma by the pineal gland. Tryptophan is an essential amino acid and it should be taken together with foods. Tryptophan, at pinealocytes, is hydroxylated to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase. 5-hydroxytryptophan is hydroxylated to serotonin. And then serotonin turns into N-acetyl serotonin by the enzyme N-acetyl transferase and that turns into melatonin (N-acetyl-5-methoxytryptamine) by the enzyme hydroxyindole-O-methyl transferase. Melatonin passes quickly to adjacent capillary vessels, not stored in the pineal gland as shown Fig 2 [11,12].

Melatonin displays high lipid and water solubility which facilitates passage across cell membranes. As no pineal storage of melatonin is available, the plasma hormone profile faithfully reflects the pineal activity. The secretion occurs at night, with maximum plasma levels around 03:00–04:00 A.M., varying with chronotype, whereas diurnal levels are undetectable, or low in rested subjects. Nearly 70% of melatonin binds to albumin in the plasma. Most of it is metabolised in liver and little in kidney [13].

Nearly 70% of melatonin binds to albumin in the plasma. Most of it is metabolised in liver and little in kidney (h.h...). In addition to the many beneficial effects, it has potent radical scavenger (effective on OH⁻, O₂⁻, peroxy radical, singlet oxygen and peroxy nitrite anion) and antioxidant (SOD, glutathione peroxidase, glutathione reductase, glucose-6-phosphate

dehydrogenase stimulation and NOS inhibition) properties. Melatonin has more potent free radical scavenging properties against OH⁻ from all known antioxidants. Since the functions of the pineal gland and melatonin levels decrease with aging, these circumstances lead to decrease and increase the diseases, to decrease antioxidant capacity and the diseases associated with an increased oxidative stress (Fig 3) [14,15].

Fig 2. The formation of melatonin

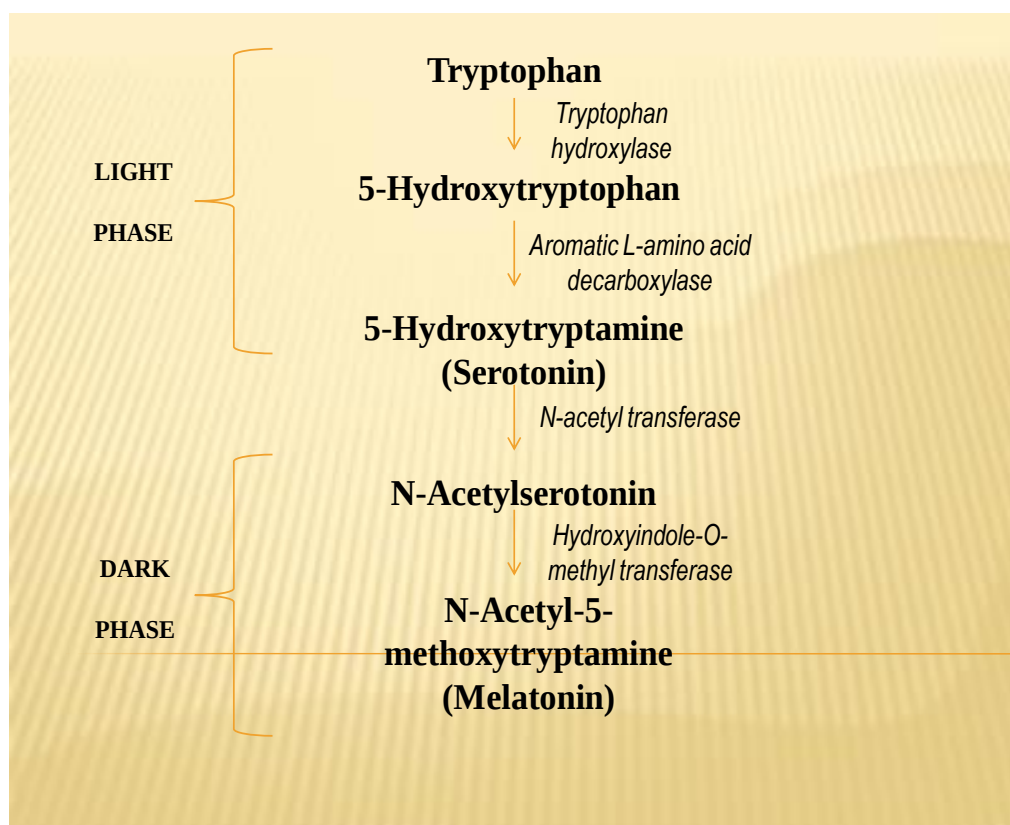
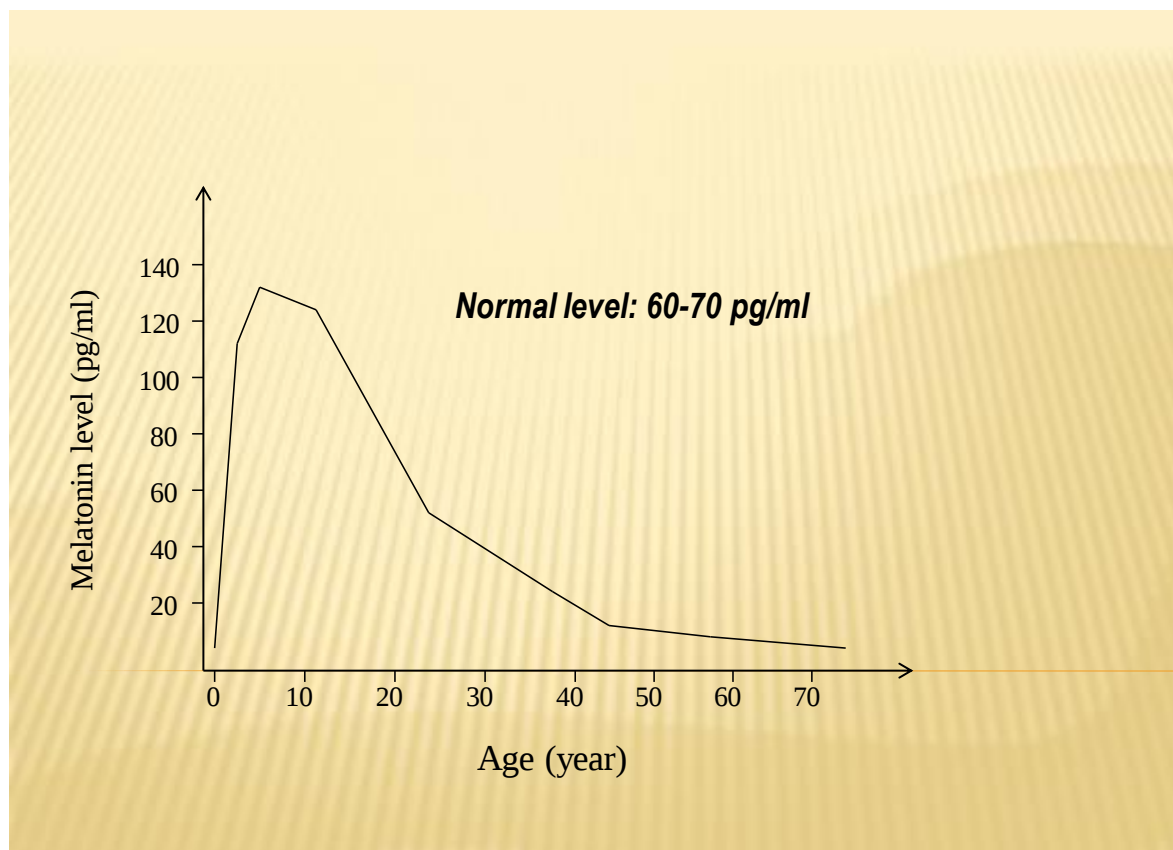


Fig 3. Melatonin secretion by age



Antioxidant Activity of Melatonin on MI/R Injury

Heart diseases, taking first place in the causes of death, and I/R induced arrhythmias have been blamed for most of the sudden deaths. Although causes of these arrhythmias are still speculative, electrophysiological abnormalities in ischemia (ion imbalance in Ca^{+2} and K^{+} levels) and excessive ROS production in reperfusion are accepted as current hypotheses. These radicals lead to cell injury resulted in membrane damage, DNA destruction, protease activation, lipid and protein peroxidation and there by apoptotic and necrotic cell damages are occur (Fig 4) [16]. It is suggested that the antioxidant impacts of melatonin can be used to protect the hearts to I/R injury [17].

Melatonin is a potent scavenger of $\text{OH}^{\cdot}$ - and peroxy radicals. The neutralizing effect of $\text{OH}^{\cdot}$ - radical is 5-fold greater than glutathione. Direct scavenging effects of melatonin on H_2O_2 and $\text{O}_2^{\cdot-}$ radicals are weak. Indirectly, it indicates some antioxidant effects by activating the enzyme GSH-Px which metabolizes the hydroxyperoxides, increasing the SOD activity which

catalyses the O_2^- radical to H_2O_2 , preventing the decline in CAT activity during oxidative stress and inhibiting the nitric oxide synthase which is responsible for the formation of NO (Fig 5) [18,19].

Fig 4. Reactive oxygen radicals

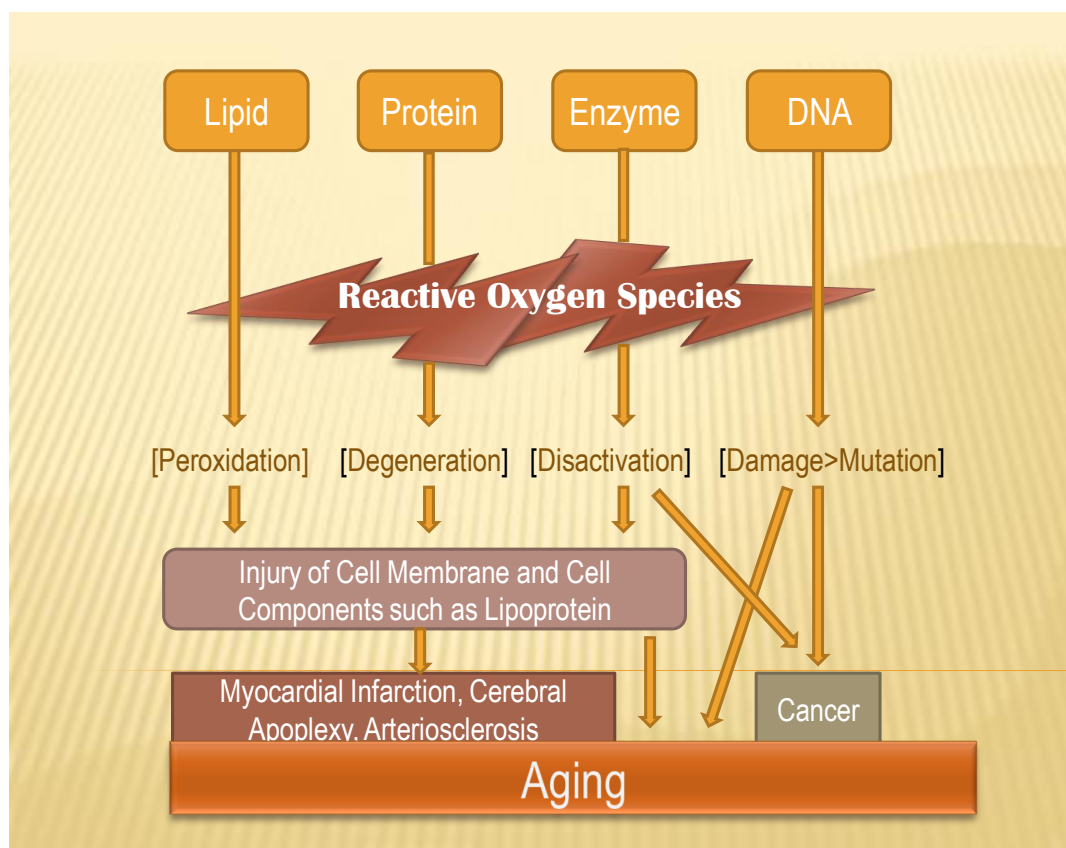
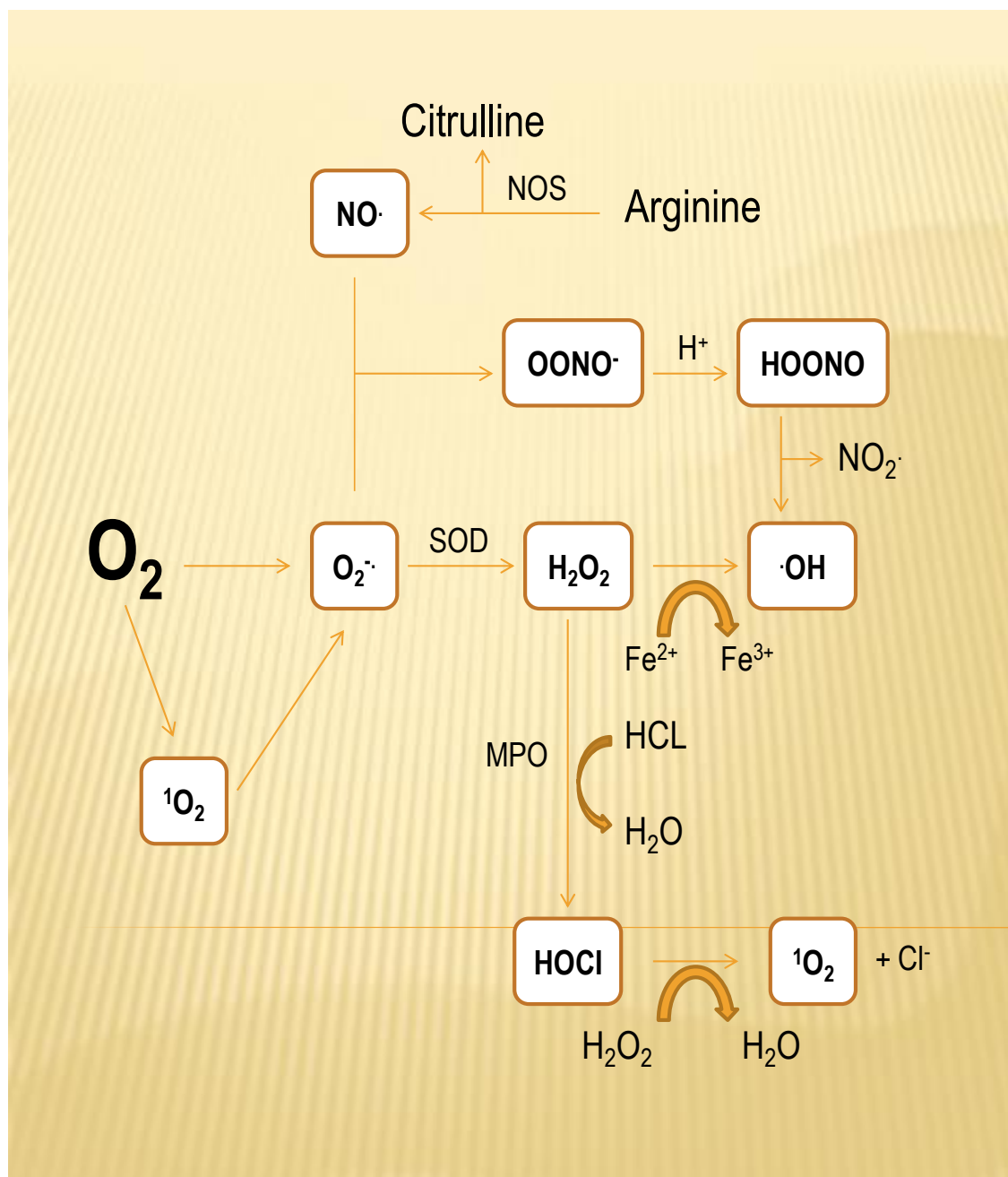


Fig 5. Cell and tissue damage by reactive oxygen species

Experimental Studies Associated with Melatonin

Due to its important properties mentioned above, a large number of studies have been and are being investigated in the world. The effects of melatonin on the cardiovascular system constitute an important part of these studies. For instance, Lee et al. [20] investigated

protective effects of melatonin on MI/R injury. This was the first in vivo study to evaluate the effects of melatonin on MI/R injury in anesthetized rats. They reported that melatonin administration at 10 min before left coronary artery occlusion markedly suppressed ventricular tachycardia (VT) and ventricular fibrillation (VF), while reducing the total number of premature ventricular contractions and total duration of VT and VF that occurred during the 45 min ischemic period. Pretreatment with melatonin significantly reduced superoxide anion production and myeloperoxidase activity (MPO) which was the most abundantly expressed in neutrophil granulocytes and played role in the antibacterial defense system in appropriate circumstances. Additionally, they indicated that melatonin reduced infarct size resulting from MI/R injury. Also, Ceyran et al. [21] examined impacts of high dose melatonin on MI/R injury. The left coronary artery was ligated for 20 min and then reperfused. Group A: Control, Group B: MI/R group without treatment, Group C: Melatonin administered group 30 min before ischemia and Group D: was planned as melatonin administered group immediately before reperfusion. In 20th reperfusion minutes, blood samples were taken for biochemical analyses. At the end of the study, the research team reached the following conclusions: the values of MDA, an index of lipid peroxidation, in Group A were significantly lower compared with the all groups. In both Groups C and D where melatonin treatments were used, a significantly lower level of MDAs was seen than that of Group B. The values of MPO in both Groups C and D, which received melatonin treatments, were significantly lower than in Group B. The values of SOD in Groups C and D, where melatonin treatments, were used showed significantly lower levels than Group B. There were no significant differences between Groups C and A for these levels. This study demonstrated that high dose melatonin protected the rat heart against MI/R injury by scavenging the ROS.

To study the effects of melatonin on ischemic preconditioning, Genade et al. [22] administered melatonin at a pharmacological dose (50 μ M) to male Wistar rats. Isolated, perfused, working hearts were subjected to 1x5 min (5 min global ischemia and 5 min reperfusion) or 3x5 min (three episodes of 5 min global ischemia, interspersed with 5 min reperfusion) ischemic preconditioning protocols, in the presence or absence of melatonin, followed by 20 min global ischemia and 30 min reperfusion. The study results indicated that free radicals played an important role in the cardiac injury which was suppressed by a multicycle (3x5 min) ischemic preconditioning protocol and that this free radical induced cardiac damage could be attenuated by a potent scavenger such as melatonin. Melatonin's

actions on the perfused heart were dependent on the time of administration. If added during reperfusion only, melatonin was cardioprotective, while when administered before and during an ischemic preconditioning protocol it abolishes protection on the basis of this hypothesis.

Zhongyi et al. [23] assessed the ability of melatonin to protect against MI/R injury in mice deficient in glutathione peroxidase 1 (Gpx1). They subjected mice hearts to 40 min of global ischemia in vitro followed by 45 min of reperfusion, and then MI/R injury was exacerbated in mice deficient in Gpx1. Pretreatment of mice with melatonin for 30 min protected the hearts of both groups of mice against I/R injury. In another set of experiments, mice hearts were subjected to 50 min of left anterior descending ligation followed by reperfusion, melatonin pretreatment was able to reduce the infarct size /area at risk as well as the infarct size /left ventricle in both groups of mice. They demonstrated that melatonin could protect the heart against I/R injury. There was significantly more apoptosis and protein nitration in hearts from Gpx1^{-/-} (deficient) mice than Gpx1^{+/+} (not deficient) mice, and melatonin attenuated apoptosis and protein nitration in both groups of mice. According to the results of their study mice deficient in Gpx1 exhibit increased apoptosis in response to I/R injury and the cardioprotective function of melatonin was independent of Gpx1.

Dobsak et al. [24] aimed to reveal the effects of melatonin in the model of isolated and perfused working rat heart, to evaluate the antioxidant capacity of melatonin and to analyse the extent of apoptosis inhibition by melatonin. The rats were divided into four groups. Group A received 50 μ M of melatonin 3 min before global ischemia and throughout the reperfusion (45 min). Group B underwent 30 min of global ischemia and 45 min of reperfusion. Group C was control and only perfused with a medium which is an oxygenated Krebs-Henseleit buffer (KHB). Group D was perfused with KHB with 50 μ M of melatonin throughout the experiment without I/R period. MDA concentrations were evaluated from heart homogenate to determine the severity of oxidative damage. Treatment by melatonin resulted in a significant improvement of hemodynamic parameters and a reduction of postischemic arrhythmias during reperfusion. Melatonin exhibited a significant dose-dependent protective effect against peroxyl radical and also reduced significantly the level of lipoperoxidation.

Semira et al. [25] demonstrated that melatonin served as a potent inhibitor of MPO under physiological-like conditions. Evidence suggested that MPO-mediated reactive oxidants could promote protein nitration, lipid peroxidation, amino acid chlorination and the thiol nitrosylation.

These species could trigger various inflammatory events affecting immune defenses, atherosclerosis, asthma and arthritis. Thus inhibition of MPO could be attractive targets in the development of biomarkers and treatment of various conditions such as atherosclerotic and cardiovascular diseases. Melatonin binding modulates the formation of MPO intermediates and their decay rates. The presence of chlorine enhanced the affinity of MPO toward melatonin, which switches the enzyme activity from peroxidation to catalase-like activity. In conclusion, they revealed that the affinity of MPO for melatonin was very high under physiological conditions. MPO may be acting as a chelator for melatonin and limiting its bioavailability and function.

Our Clinic's Experience on Melatonin in The Cardiovascular System

Melatonin plays an important role in the regulation of various body functions including circadian sleep rhythms, blood pressure, oncogenesis, retinal function, seasonal reproduction and immunity (Table 1). Because of these important properties, as well as many different clinics, a significant number of studies have been done in our clinic regarding with melatonin.

Table 1: Some beneficial effects of melatonin.

Effects of melatonin

- Corrects disrupted circadian rhythms
- Improves sleep disorders and jet lag
- Regulates immunomodulatory cytokines
- Potent inhibitor of apoptosis
- Oncostatic properties
- Enhances efficacy of cancer chemotherapy and reduces its toxicity
- Protective effects on atherosclerosis
- Decreases blood pressure
- Anti-anginal and anti-ischemic effects
- Prevents I/R-induced cardiac infarct size
- Protective and preventive effects on contrast-induced nephropathy
- Modulates neuronal activity
- Anticonvulsant activity
- Improves migraine and cluster headache

To investigate the in vivo effects of melatonin at physiological concentrations and to provide the decreased endogen melatonin levels, Sahna et al. [26] applied pinealectomy (Px) into the

rats. They occluded the coronary artery for 7 min and following reperfusion for 7 min. It was determined that the degree of cardiac arrhythmias and mortality were significantly high in the rats with Px when compared with control group. In the following study [27]; the coronary artery was occluded for 30 min and reperfusion for 120 min and revealed that infarct size in the rats with Px was significantly enlarged. All of these findings were suggested that endogenous melatonin levels had protective effects to the heart during I/R. These studies indicated that melatonin given externally (4 mg/kg) also significantly reduced damage in Px rats.

Sahna et al. [15] designed a study to investigate the effects of melatonin on MI/R-induced infarct size and oxidative changes. They used male wistar rats in their study and divided them into three groups; Control, I/R+vehicle and I/R+melatonin. Vehicle or melatonin (10 mg/kg) was administered by intravenous injection 10 min before ischemia. The main coronary artery was occluded for 30 min and reperfusion for 120 min before experiment was terminated. Infarct size, expressed as the percentage of the risk zone, was found significantly to be greater in I/R group than in the melatonin-treated group. MDA levels were significantly higher; however, GSH levels were lower in the I/R group than in the control group. Melatonin significantly reduced the MDA values and increased the GSH levels. The results indicated that melatonin improved the antioxidant capacity of the heart and attenuated the degree of lipid peroxidation after MI/R.

Discussion

It is well known that after MI, free radicals, cytokines and antioxidants play a major role in myocardial damage. Early reperfusion of the ischemic area is important in maintaining of the myocardial tissue. A delay in the reperfusion often results in injury to the myocardial cells, which has been termed reperfusion injury. Oxidative stress contributes to MI/R injury and oxygen-derived free radicals can cause damage. Lipid peroxidation as a free radical-generating system has been suggested to be closely related to I/R-induced tissue damage, and MDA is a good indicator of the rate of lipid peroxidation [16]. GSH is an antioxidant, preventing damage to important cellular components caused by ROS. Tissue GSH is an indicator of postischemic injury. GSH scavenges O₂⁻ protects protein thiol groups from oxidation. The depletion in GSH levels during I/R was probably due to its consumption during oxidative stress. Another important antioxidative enzyme, MPO, can also result an increase in I/R injury. MPO plays a fundamental role in oxidant production by neutrophils.

Neutrophil-specific enzyme, MPO, activity can be assessed during I/R and can be commented. In some studies, it had been indicated that reperfusion causes higher enzyme activity than ischemia [28,29].

Melatonin was discovered nearly 50 years ago. From that date to day, many researches have been done and are being done. Melatonin plays an important role in the whole body. It is a free radical scavenger that has the ability to neutralize the toxicity of the hydroxyl radical, the singlet oxygen, and the possibly the peroxy radical and the superoxide anion. As an antioxidant, melatonin protects DNA, membrane lipids and cytosolic proteins from oxidative damage.

Beside its direct free radical scavenging actions, melatonin activates antioxidant enzymes. Some of melatonin's antioxidant actions probably derive from its stimulatory effects on SOD, GSH-Px, GSH-Rd, and glucose-6-phosphate dehydrogenase and its inhibitory action on inducible nitric oxide synthase [18]. Melatonin reduces the I/R damage to the other organs such as the kidney [14], testis [30] and liver [31] in rats. Similarly, melatonin protects against cardiotoxicity induced by chemotherapeutic drugs, which are often in cardiac tissue [32].

In this review, we summarized the effects of antioxidant property of melatonin associated with MI/R injury. According to the knowledges mentioned above, it can be possible to say that oxidative stress occurs during MI/R and melatonin administration exerts an infarct size limiting effect, antiapoptotic, antiarrhythmic effects and enhances antioxidant activity of the heart. More detailed clinical studies are needed to elucidate the exact mechanism(s) of melatonin.

Conflict of Interest Statement

All authors declare that they have no conflicts of interest.

References

1. Zimmerman BJ, Granger DN. Reperfusion injury. Surg Clin North Am. 1992;72(1):65-83.
2. Wilhelm J. Metabolic aspects of membrane lipid peroxidation. Acta Univ Carol Med Monogr. 1990;137:1-53.
3. Harman AW, Maxwell MJ. An evaluation of the role of calcium in cell injury. Annu Rev Pharmacol Toxicol. 1995;35:129-44.

4. Kukreja RC, Hess ML. The oxygen free radical system: from equations through membrane protein interactions to cardiovascular injury and protection. *Cardiovasc Res.* 1992;26(7):641–55.
5. Jennings RB, Ganote GB, Kloner RA, Whalen DA, Hamilton DG. Explosive swelling of myocardial cells irreversibly injured by transient ischemia. In: *Recent Advances in Studies on Cardiac Structure and Metabolism. Vol 6: Pathophysiology and Morphology of Myocardial Cell Alteration.* Baltimore, Md: University Park Press. 1975:405-13.
6. Dhallaa NS, Elmoselhia AB, Hatac T, Makino N. Status of myocardial antioxidants in ischemia–reperfusion injury. *Cardiovasc Res.* 2000;47(3):446-56.
7. Ceconi C, Cargnoni A, Pasini E, Condorelli E, Curello S, Ferrari R. Evaluation of phospholipid peroxidation as malondialdehyde during myocardial ischemia and reperfusion injury. *Am J Physiol.* 1991;260(4 Pt 2):1057-61.
8. Brezinski A. Mechanisms of disease: Melatonin in humans. *N England J Med.* 1997;336:186–95.
9. Reiter RJ. The melatonin rhythm: Both a clock and a calendar. *Experientia.* 1993;49(8):654-64.
10. Tan DX, Manchester LC, Hardeland R, Lopez Burillo S, Mayo JC, Sainz RM, Reiter RJ. Melatonin: a hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. *J Pineal Res.* 2003;34(1):75-8.
11. Sprenger J, Hardeland R, Fuhrberg B, Han SZ. Melatonin and other 5-methoxylated indoles in yeast: Presence in high concentrations and dependence on tryptophan availability. *Cytologia.* 1999;64:209-13.
12. Hardeland R, Poeggeler B. Non-vertebrate melatonin. *J. Pineal Res.* 2003;34(4):233-41.
13. Reiter RJ. The aging pineal and its physiological consequences. *BioEssays.* 1992;14(3):169-75.
14. Parlakpınar H, Sahna E, Ozer MK, Ozugurlu F, Vardi N, Acet A. Physiological and pharmacological concentrations of melatonin protect against cisplatin-induced acute renal injury. *J Pineal Res.* 2002;33(3):161-6.
15. Sahna E, Parlakpınar H, Turkoz Y, Acet A. Effects of melatonin on myocardial ischemia-reperfusion-induced infarct size and oxidative stress. *Physiol Res* 2005;54(5):491-5.
16. Parlakpınar H, Orum MH, Sagir M. Pathophysiology of myocardial ischemia reperfusion injury: A review. *Med Science.* 2013;2(4):935-54.
17. Parlakpınar H, Sahna E, Ozgen U, Mizrak B, Acet A. Protective role of melatonin on myocardial ischemia-reperfusion-triggered apoptosis. *Proceedings of the Turkish Pharmacological Society, 17th National Congress of Pharmacology, 1st Clinical Pharmacology Symposium, Joint Meeting of the Turkish & Dutch. Pharmacological Societies; 17-21 October 2003. Antalya, Turkey.*
18. Reiter RJ, Tan DX. Melatonin: A novel protective agent against oxidative injury of the ischemic-reperfused heart. *Cardiovascular Research.* 2003;58(1):10-9.

19. Reiter RJ, Tan DX, Kim SJ, Wenbo QI. Melatonin as a pharmacological agent against oxidative damage to lipids and DNA. *Proc West Pharmacol Soc.* 1998;41:229-36.
20. 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(2):72-80.
21. Ceyran H, Narin F, Narin N, Akgun H, Ceyran AB, Ozturk F, Akcali Y. The effect of high dose melatonin on cardiac ischemia- reperfusion Injury. *Yonsei Med J.* 2008;49(5):735-41.
22. Genade S, Ytrehus K, Lochner A. Melatonin prevents cardioprotection induced by a multi-cycle ischaemic preconditioning protocol in the isolated perfused rat heart. *Cardiovasc J S Afr.* 2006;17(5):239-44.
23. Chen Z, Chua CC, Gao J, Chua K-W, Ho Y-S, Hamdy RC, Chua BHL. Prevention of ischemia/reperfusion-induced cardiac apoptosis and injury by melatonin is independent of glutathione peroxidase 1. *J Pineal Res.* 2009;46(2):235-41.
24. Dobsak P, Siegelova J, Eicher JC, Jancik J, Svacinova H, Vasku J, Kuchtickova S, Horky M, Wolf JE. Melatonin protects against ischemia-reperfusion injury and inhibits apoptosis in isolated working rat heart. *Pathophysiology.* 2003;9(3):179-87.
25. Galijasevic S, Abdulhamid I, Abu-Soud HM. Melatonin is a potent inhibitor for myeloperoxidase. *Biochemistry.* 2008;47(8):2668-77.
26. 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(3):194-8.
27. Sahna E, Acet A, Ozer MK, Olmez E. Myocardial ischemia-reperfusion in rats: Reduction of infarct size by either supplemental physiological or pharmacological doses of melatonin. *J Pineal Res.* 2002;33(4):234-8.
28. Kacmaz A, 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(9):744-50.
29. Parlakpinar H, Ozer MK, Sahna E, Acet A. Attenuation of ischemiareperfusion-induced myocardial infarct size in rats by aminoguanidine. 6th Congress of the European Association for Clinical Pharmacology and Therapeutics, 24-28 June 2003. Istanbul, Turkey.
30. 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(3):365-72.
31. Jahovic N, Cevik H, Sehirli AO, Yegen BC, Sener G. Melatonin prevents methotrexate-induced hepatorenal oxidative injury in rats. *J Pineal Res.* 2003;34(4):282-7.
32. 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(4):257-61.