Ischemic Heart Disease: Its association with reactive oxygen species:

Ischemic Heart Disease (IHD) [also known as myocardial ischaemia] is characterized by reduced blood supply to the heart muscle, usually due to coronary artery disease (atherosclerosis of the coronary arteries). Its risk increases with age, smoking, hypercholesterolemia, diabetes, and hypertension, and is more common in men and those who have close relatives with IHD. Acute coronary occlusion resulting in IHD is the most common cause of death in most western countries and its occurrence is now on the rise in the developing nations as well. According to the World Health Organization (WHO), it will be the major cause of death in the world by the year 2020 [19].

Fig. 1: Development of myocardial infarction
Ischemic heart disease may be present with any of the following problems:

- **Stable angina or angina pectoris** whose symptoms include characteristic chest pain on exertion, in cold weather or emotional situations and decreased exercise tolerance

  ![Radiation of anginal pain into the left arm.](image)

  Fig. 2: Radiation of anginal pain into the left arm.

- **Unstable angina or myocardial infarction** which presents itself as acute chest pain or other symptoms at rest or rapidly worsening angina (acute coronary syndrome).

- **Heart failure** whose symptoms include difficulty in breathing or swelling of the extremities due to weakness of the heart muscle

  The medical history distinguishes between various alternative causes for chest pain (such as dyspepsia, musculoskeletal pain, pulmonary embolism). As part of an assessment of the three main presentations of IHD, risk factors are addressed. These are - age, male sex, hyperlipidaemia (high cholesterol and high fats in the blood), smoking, hypertension (high blood pressure), diabetes, and the family history

  Ischemic heart disease can be diagnosed with an electrocardiogram, blood tests (cardiac markers), cardiac stress testing or a coronary angiogram. Depending on the symptoms and risk, treatment of patients may be carried out with medication, percutaneous coronary intervention (angioplasty) or coronary artery bypass surgery (CABG). Despite effective reperfusion of epicardial coronary arteries by percutaneous coronary intervention or thrombolysis, there still remain higher chances of morbidity
and mortality [20]. Infarct size is also an important determinant of the short- and long-term outcome after acute myocardial infarction [21].

Although beneficial in terms of myocardial salvage, reperfusion itself may contribute to additional damage of the myocardium, due to the combined processes known as “ischemia-reperfusion injury” [I/R]. The pathogenesis of myocardial ischemia-reperfusion injury is a multi-factorial process involving the interaction of multiple mechanisms.

Reactive oxygen species play a critical role in the pathogenesis of various diseases including cardiovascular injury associated with circulatory disturbance. It is estimated that approximately 5% of the oxygen consumed by normal tissues are transformed into ROS which include superoxide anion free radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) (a non-radical ROS), and free hydroxyl radical (OH) [5].

![Fig. 3: The cellular pathways for the generation and scavenging of reactive oxygen species (ROS)](image)
Recent studies have indicated the involvement of ROS in myocardial ischaemia [3]. ROS have been shown to exert direct inhibitory effect on myocardial function both in vivo and in vitro including cellular loss of K⁺, depletion of high energy phosphates, elevated intra-cellular Ca²⁺ concentration, loss of systolic force development, a progressive increase in diastolic function and arrhythmias [1].

![Diagram](attachment:image.png)

**Calcium hypothesis**

- Na → Ca
- H → Ca

**Free-radical hypothesis**

- O₂-derived FRs → Ca

**Ischemia/reperfusion**

- Reversible injury
  - Hibernation
  - Stunning
- Irreversible injury
  - Necrosis
  - Apoptosis

- Viable myocytes decreased function
- Non viable myocytes decreased function

- Revascularization → Restored myocardial function

**Fig. 4 (A) and (B): The cellular mechanisms involved in the pathogenesis of myocardial ischemia / reperfusion (I/R) injury**

- Myocardial ischemia / reperfusion → Reactive Oxygen Species → Cytokines → NF-κB
- Endothelial Cell Adhesion Molecules
- Leucocyte/Endothelial Cell Interaction
- Myocardial Cell Injury
**Melatonin**

Systematic (IUPAC) name: 
N-[2-(5-methoxy-1H-indol-3-yl)ethyl] ethanamide  
Formula: C_{13}H_{16}N_{2}O_{2}  
Mol. mass: 232.278 g/mol  
Metabolism: Hepatic via CYP1A2 mediated 6-hydroxylation  
Excretion: Urine  
Average physiological plasma levels: 10-60pg/mL

![Structure of melatonin](image)

**Melatonin (N-acetyl-5-methoxy tryptamine),** a tryptophan derivative, was first isolated from bovine pineal glands and was structurally identified in 1958 by Aaron Lerner [21]. It is a naturally occurring compound found in microbes, plants and animals including humans.

In mammals, melatonin is secreted into the blood by the pineal gland in the brain. The pineal production of melatonin in vertebrates exhibits an unambiguous circadian rhythm with its peak near the middle of scotophase and basal levels during the photophase. Known as the "hormone of darkness", it is secreted in darkness in both day-active (diurnal) and night-active (nocturnal) animals.

![Diurnal rhythm of melatonin secretion in](image)
Melatonin was found to be a sleep promoter [22, 23], a chemical signal of light and darkness (Zeitgeber) as well as a regulator of photoperiod-dependent seasonal reproduction in some vertebrates [7, 22]. The pineal melatonin production in mammals including humans changes with age and its amount waning with the advancement of age (Fig. 6).

**Biosynthesis of melatonin:**

The amino acid L-tryptophan is considered to be the primary precursor of melatonin biosynthesis. Melatonin is biosynthesized through several enzymatic steps including tryptophan 5-hydroxylation, decarboxylation, N-acetylation and O-methylation. Alternatively, but at lower flux rates, melatonin can also be formed via O-methylation of serotonin and subsequent N-acetylation of 5-methoxytryptamine or by O-methylation of tryptophan followed by decarboxylation and N-acetylation [14].

![Fig. 7: The synthesis and catabolism of melatonin. AMK, N1-acetyl-5-methoxykynurenine; AA-NAT, arylalkylamine N-acetyltransferase; TPH, tryptophan hydroxylase; AAAD, aromatic amino acid decarboxylase; AAA, aryl acylamidase; NAT, N-acetyltransferase; HIOMT, hydroxyindole O-methyltransferase](image-url)
The hydroxy indole-O-methyl transferase (HIOMT) is one of the major enzymes regulating melatonin biosynthesis. There are two main pathways in the catabolism of melatonin. About 60% of melatonin is hydroxylated to 6-hydroxymelatonin, which undergoes further conjugation to form either 6-sulfomelatonin or 6-hydroxymelatonin glucuronide. Furthermore, about 15% is metabolized to the N1-acetyl-5-methoxy-kynurenine (AMK), while about 25% of melatonin remains unchanged. All the metabolites are excreted into urine and, as in melatonin synthesis there is a circadian rhythm in the excretion, with higher rates during darkness [24].

Originally, melatonin was believed to be synthesized exclusively in the pineal gland of vertebrates. However, melatonin of extra-pineal origin has also been identified [25, 26]. It is produced by a variety of peripheral cells such as bone marrow cells, ganglionic cells of retina, lymphocytes, gastric mucosal cells and epithelial cells [14]. The biosynthetic pathways in these cells have been established [14]. Usually, the melatonin concentration in these cells is much higher than that found in the blood but it does not seem to be regulated by the photoperiod [14]. It is speculated that the local melatonin synthesized in the tissues is consumed up as a protective measure against oxidative stress [14]. Melatonin has been shown to reach and bind to melatonin receptors in the brains of chicks that ingested a plant feed such as rice reported to be rich in melatonin [27]. Further, consumption of walnuts has been shown to elevate plasma melatonin level in humans [14].

The well-documented effects of melatonin and its metabolites as antioxidants have shown that they protect cells, tissues and organs from oxidative damage induced by ROS as well as from nitrogen-based reactants [28, 29]. Melatonin is particularly effective in neutralizing the hydroxyl radical (•OH) which attacks DNA, proteins and lipids leading to a variety of disorders [13, 30]. Melatonin also detoxifies superoxide anion free radical (O_2•^-) [31], nitric oxide (NO•), peroxynitrite anion (ONOO•^-) [32], hypochlorous acid (HOCI) [33], the haemoglobin oxoferryl radical [34], ABTS+ cation radical and possibly the peroxyl radicals (LOO•) [33], all of which...
cause cell damage [35]. In addition, melatonin inhibits inducible nitric oxide synthetase (iNOS) [36] and stimulates several antioxidant enzymes [37]. Additionally, it increases the efficiency of the electron transport chain and, as a consequence, likely reduces electron leakage and the generation of free radicals [38]. Due to its antioxidative actions, melatonin protects against heavy metals [39] and other toxic agents and it works synergistically with exercise to improve stroke volume [40].

Fig. 8: Melatonin and some of its metabolites
Melatonin receptors:
Many biological effects of melatonin are produced through activation of melatonin receptors. The first melatonin receptor gene, expressed in *Xenopus laevis* melanophores, was cloned in 1994 [41]. After that many melatonin receptors and receptor fragments have been cloned from different animal classes. The length of melatonin receptor proteins is 346-420 amino acids and their molecular weights are 39-47 kDa [42]. Since melatonin receptors are located in the cell membrane, melatonin regulates the function of the cell through G-protein-regulated effectors. Based on their DNA and amino acid sequences, the melatonin receptors can be divided into three subtypes. Two of these, MT1 and MT2, are expressed in mammals, and the third, Mel1c, is expressed in birds, amphibians and fish [45]. Two of these melatonin receptors (MT1 and Mel1c) has similar pharmacological specificity: 2-iodomelatonin > melatonin > 6-chloromelatonin > 6-hydroxymelatonin > N-acetyl-5-hydroxytryptamine > serotonin. MT2 receptors differ only in that the affinity of 2-iodomelatonin, melatonin and 6-chloromelatonin is equal for this receptor [43].

Melatonin as an antioxidant:
Tan et al. in 1993 have shown for the first time that melatonin does possess free radical scavenging ability in *in vitro* system [13]. The ability of melatonin to serve as an antioxidant at both physiological as well as pharmacological concentrations *in vivo* in humans and different experimental models of oxidative stress have been demonstrated [31]. Some of the recent reports have shown that pharmacological doses of melatonin protects against stress- and drug-induced gastric ulceration in experimental rats through its antioxidant mechanism(s) [25]. Melatonin can function as a
pervasive and powerful antioxidant with a particular role in the protection of
nuclear and mitochondrial DNA [39]. The antioxidant properties of melatonin
and its possible regulatory effects on ROS production and redox signaling
have been proposed to play a key role in antagonizing the mitochondrial
pathway of apoptosis [40]. In the recent years, several findings support the
antioxidant effect as well as a direct role of melatonin in mitochondrial
homeostasis [44]. This latter action of melatonin may contribute to
melatonin’s protective effects in degenerative disorders such as Parkinson’s
disease, Alzheimer’s disease, epilepsy, aging, ischemia-reperfusion and
sepsis, all of which involve mitochondrial dysfunction as a primary or
secondary cause of the disease [45]. Melatonin’s ability to provide protection
to the heart has been shown in different models of oxidative stress [46-48]
and is currently an emerging area of research.
Isoproterenol

Systematic (IUPAC) name: 4-[1-hydroxy-2-(isopropyl amino) ethyl] benzene-1,2-diol
Formula: C_{11}H_{17}NO_{3}
Mol. Mass: 211.258 g/mol

Route of administration: intravenous, oral, intranasal, subcutaneous, or intramuscular

LD_{50} of ISOPROTERENOL injected subcutaneously in the normal RAT: 680 mg/kg BW.

Fig. 10: Structure of Isoproterenol (ISO)

Isoproterenol (ISO) is a synthetic, sympathomimetic catecholamine adrenergic receptor (β1 β2 non-selective) agonist. It is structurally similar to adrenaline. The isopropyl amine group in isoproterenol makes it selective for β receptors. The free catechol hydroxyl groups keep it susceptible to enzymatic metabolism.

Effects on the cardiovascular system:

The effects of isoproterenol on the cardiovascular system (non-selective) relate to its actions on cardiac β_{1} receptors and β_{2} receptors on skeletal muscle arterioles. Isoproterenol has positive inotropic and chronotropic effects on the heart. In skeletal muscle arterioles, it produces vasodilatation. The inotropic and chronotropic effects of ISO elevate systolic blood pressure, while its vasodilatory effects tend to lower diastolic blood pressure.
Isoproterenol can produce an elevated heart rate (tachycardia), which predisposes the patients to cardiac dysrhythmias. Deaths have been reported following excessive use of ISO inhalation preparations and the exact cause is unknown. Cardiac arrest was noted in several instances.

Administration of isoproterenol has been reported to produce gross and microscopic infarct in rat heart [7]. Studies have shown that the pathophysiological changes that take place in rat heart following myocardial infarction induced by ISO are comparable to the changes taking place after myocardial infarction in humans [7]. Isoproterenol (ISO), upon oxidation, produces quinones which react with oxygen to produce $O_2^*$- and hydrogen peroxide ($H_2O_2$). The production of $O_2^*$- results in the liberation and reduction of iron from tissue ferritin [28] as well as the secondary formation of $H_2O_2$ and the $•OH$ [13]. Since iron and $•OH$ are both initiators of lipid

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**Fig. 11: Isoproterenol signalling during cardiac contraction.** AC, adenylate cyclase; β-AR, β-adrenergic receptor; β-ARK, β-adrenergic receptor kinase; DHPR, dihydropyridine receptor; FKBP12.6, FK-506 binding protein (calstabin2); PKA, protein kinase A; PLB, phospholamban; RyR2, ryanodine receptor; SERCA2a, sarco-endoplasmic reticulum Ca2+-ATPase2.
peroxidation (LPO) [30], an important biomarker of oxidative stress, oxidative stress might be an important determinant of myocardial injury.

**Fig. 12: Actions of Isoproterenol on heart**

**Role of melatonin in myocardial ischemia**

The role of ROS in ischemic heart disease and the protective effect of various antioxidants have been studied extensively [5]. The pineal hormone, melatonin, is known to be a highly efficient scavenger of free radicals, even more efficient than the well-known antioxidants vitamin E, ascorbic acid and glutathione (GSH). Because of its amphiphilicity (i.e., solubility both in lipids and water), melatonin can easily enter every cellular compartment and actively reduces oxidative damage to macro-molecules such as nuclear DNA [49], lipids [50] and proteins [51]. Melatonin is able to remove directly many ROS, among them the hydroxyl radical (·OH), the most reactive and toxic of the free radicals. Melatonin’s interaction with free radicals forms metabolites
that have been reported to be effective free radical scavengers [14].
Melatonin also functions as an indirect antioxidant by acting synergistically
with other antioxidants and stimulating the synthesis of the major
antioxidant enzymes including SOD, CAT and GPx [50].

A number of researchers have shown that the cardio-protective
ability of melatonin might be exerted through its radical scavenging property
[5]. However, possibility of involvement of other mechanism(s) cannot be
ruled out. A majority of current research focuses on melatonin’s ability to
scavenge free radicals in different models of oxidative stress [15-17]. 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 [2]. 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 [52]. The results of
Kacmaz et al. [53] also confirmed that melatonin has a protective effect on
I/R-induced oxidative cardiac damage as well as antiinflammatory effect.
The protective effects of melatonin probably occurred, in part, due to the
scavenging of any highly reactive •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. 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 [54]. Collectively, these protective actions of melatonin may also have potential clinical applicability for individuals with cardiovascular disease including those treated with angioplasty [55].

Isoproterenol-induced myocardial ischemic model is simple in that it generates free radicals through its metabolism causing ischemic stress on the myocardium. Understanding the protective role of melatonin in myocardial ischemia is an interesting task since it is a highly conserved natural molecule present both in plants and animals and its pharmacological doses have been found to be non-toxic.