CHAPTER 2

LITERATURE REVIEW

2. REVIEW OF LITERATURE

2.1. ETIOLOGY OF CORONARY HEART DISEASE (CHD)

The risk factors for the development of CHD and angina pectoris are genetic predisposition, age, sex and a series of reversible risk factors: The most important factors include high fat and cholesterol rich diets, lack of exercise, inability to retain normal cardiac function under increased exercise tolerance due to tobacco, smoking, excessive alcohol drinking, carbohydrate, fat metabolic disorders, diabetes, hypertension obesity and the use of drugs that produce vasoconstriction or enhanced oxygen demand. The increased cholesterol levels caused by the consumption of a diet rich in saturated fat stimulates the liver to produce cholesterol; a lipid needed by all cells for the synthesis of cell membranes and in some cells for the synthesis of other steroids. This is the principal reversible determinant of risk of heart disease. Low density lipoproteins (LDLs, also referred to as "bad" cholesterol) transport cholesterol from liver to other tissues whereas high density lipoproteins (HDLs, also referred as "good" cholesterol) transport cholesterol from tissues back to the liver to be metabolized. Triglycerides are transported from the liver to the tissues mainly as very low density lipoproteins (VLDLs). VLDLs are the precursors of the LDLs. The LDLs are characterized by high levels of cholesterol; mainly in the form of highly insoluble cholesteryl esters.
However, there is a strong relationship between high LDL levels and coronary heart disease and a negative correlation between HDL and heart disease. In general, for people who have total cholesterol levels lower than 200mg/dL, heart attack risk is relatively low. If the total cholesterol level is 240 mg/dL, the person has twice the risk of heart attack as someone who has a cholesterol level of 200 mg/dL. The cholesterol levels of 240 mg/dL are considered high and the risk of coronary heart disease.

The reduction of LDL cholesterol levels lower the cardiovascular risk. During the last few years, there has been reliable evidence that coronary artery disease is a complex genetic disease. In fact, a number of genes associated with lipoprotein abnormalities and genes influencing hypertension, diabetes, obesity, immune and clotting systems play important roles in atherosclerotic cardiac disorders. Researchers have identified genes regulating LDL cholesterol, HDL cholesterol and triglyceride levels based on common genetic variation. Many genes linked to CAD are involved in determining how the body removes

low density lipoprotein (LDL) cholesterol from the bloodstream. If LDL is not properly removed, it initiates ischemia-reperfusion syndrome which is defined as myocardial injury caused by the restoration of coronary flow. This phenomenon has a complex pathophysiology and results in a paradoxical reduction of the beneficial effect of myocardial reperfusion. Studies suggest that ischemia-reperfusion injury may account for up to 50% of the final size of a myocardial infarct.

The technique of reperfusion during acute myocardial infarction has led to a dramatic decrease in the morbidity and mortality associated with coronary artery disease in recent decades. The restoration of blood flow within the "golden hours" has resulted in a reduction in myocardial infarct size. Although greatly beneficial overall, the abrupt restoration of blood flow in the coronary arteries after occlusion was also, surprisingly, found to be associated with an additional and accelerated myocardial injury beyond that generated by ischemia alone, an observation first reported by Jennings *et.al.* This phenomenon has been called "ischemic-reperfusion injury".

The process has a complex pathophysiology leading to cardiomyocyte death that is distinct from that associated with ischemic injury. Because of the deleterious effects of ischemia-reperfusion injury, several treatments aiming to prevent or limit this process have been proposed. Trimetazidine (Vastarel MR) is an antianginal drug that acts by switching the energy substrate from fatty acid metabolism to glucose metabolism, thus making possible the increased formation of ATP with a decreased need for oxygen .These properties are of great potential interest to the reduction of ischemic-reperfusion injury.

2.1.1. Role of oxidative stress in cardiovascular diseases

Several lines of evidence have demonstrated that oxidative stress plays an important role in the pathogenesis and development of cardiovascular diseases including hypertension, hypercholesterolemia, diabetes mellitus, atherosclerosis, myocardial infarction, angina pectoris and heart failure (Chien, 1999; Griendling and Fitzgerald, 2003; Stocker and Keaney, 2004; Benjamin and Schneider, 2005). The susceptibility of vascular cells to oxidative stress is a function of the overall balance between the degree of oxidative stress and the antioxidant defense capability **(Fig.1).**

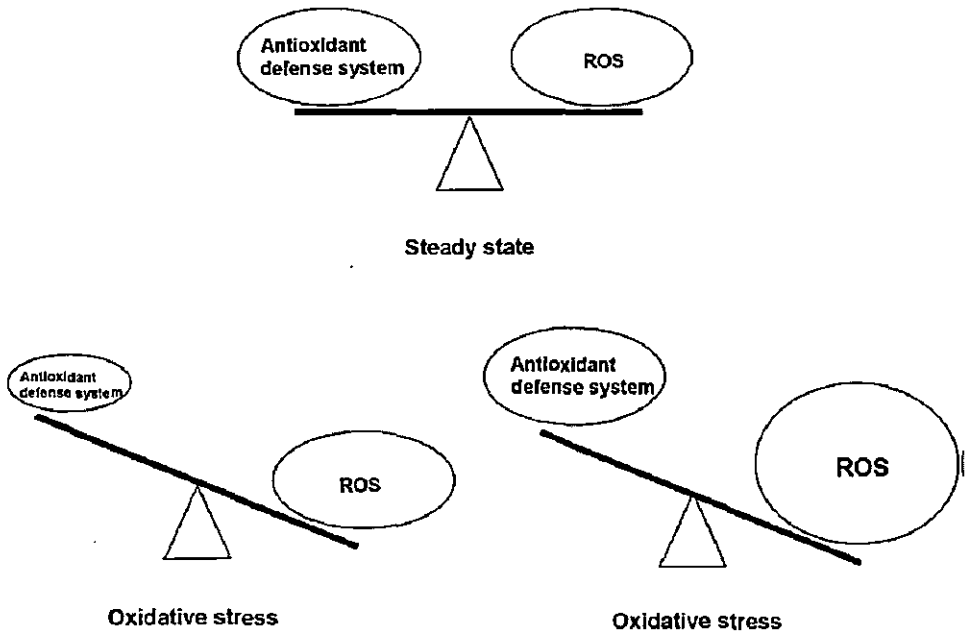

Fig.1. Steady state and oxidative stress: a balance between the degree of oxidative stress and the antioxidant defense capability. ROS indicates reactive oxygen species.

Protective antioxidant mechanisms are complex and multifactorial. The antioxidant defense systems such as Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX) and catalase(CAT) scavenge ROS in the vasculature resulting in inhibition of Nitric Oxide (NO) degradation. Although SOD rapidly converts superoxide to hydrogen peroxide, hydrogen peroxide itself is involved in vascular remodeling, inflammation, apoptosis and growth of vascular smooth muscle cells as an intracellular second messenger (Fukai *et al.,* 2000). The production of lipid peroxidation and protein oxidation induce over expression of redox genes, intracellular calcium overload and DNA fragmentation, resulting in damage of vascular smooth muscle cells, endothelial cells or myocardial cells.

2.2. PATHOPHYSIOLOGY OF ISCHEMIA-REPERFUSION INJURY

The pathophysiology of ischemia-reperfusion injury is complex. In this process, the re-oxygenation of ischemic myocardium generates a high degree of myocardial injury as a result of the generation of potent oxygen-derived free radicals. This phenomenon is known as the "oxygen paradox". The oxidative stress also reduces the bioavailability of nitric oxide which is critical to the improvement of coronary blood flow and inactivation of

superoxide radicals. Reperfusion is also associated with an abrupt increase and overload of intracellular calcium which cause hyper contracture of cardiomyocytes, leading to cell death. The activation and accumulation of polymorphonuclear neutrophils occur in the damaged myocardium and contribute to the ischemia-reperfusion injury. The neutrophils are important for the development of reperfusion injury, releasing oxygen free radicals, proteases and pro inflammatory mediators that further amplify the infiltration of neutrophils into the jeopardized myocardium. The other processes involving platelets and complement also participate in ischemia-reperfusion injury. Acting together, these effects may last hours or months after reperfusion and participate in sustained cardiomyocyte death.

The exact contribution of ischemia-reperfusion injury to infarct size is difficult to determine. However, on the basis of the observed reduction in infarct size associated with treatments preventing ischemia reperfusion injury, it is postulated that up to 50% of the final size of the myocardial infarct is linked to the ischemia reperfusion injury. The reduction of this phenomenon should provide great clinical benefit, and is therefore currently the subject of extensive experimentation.

Myocardial injury has been implicated in the pathology of peripheral vascular insufficiency (Muller *et al.,* 2002), angina (Verma *et al.,* 2002), myocardial infarction (Mc Donough *et al.,* 1999) and stroke (Oliver *et al.,* 1990). Brief intermittent periods of ischemia followed by reperfusion at a time prior to prolonged ischemia known as ischemic preconditioning or immediately after a period of ischemia before the onset of reperfusion known as ischemic post conditioning, have been shown to reduce I/R-induced myocardial injury (Murry *et al.,* 1986; Zhao *et al.,* 2003).

The basic mechanisms involved in the pathophysiology of I/R injury and the pharmacology of pre-conditioning and post conditioning are discussed hereunder.

The important consequences of ischemic reperfusion are reversible contractile dysfunction known as myocardial stunning and impairment of blood flow at micro vascular level known as no reflow with neutrophil plugging and vasoconstriction. Myocardial stunning is the contractile dysfunction of heart that persists after reperfusion despite the absence of irreversible damage and despite restoration of normal or nearly normal coronary flow (Bolli, 1990). The impairment in resynthesis of high energy phosphates, alteration in sympathetic

responsiveness, damage to collagen matrix, leukocyte activation, transient calcium overload, decreased sensitivity of myofilaments to calcium and generation of oxygen free radicals have been implicated in the pathogenesis of prolonged contractile dysfunction in myocardial stunning (Ferrari *et al.,* 1995). The ischemic myocardium reduces its metabolic needs and tends to adopt itself to survive with minimal requirements by reducing its own contractility. Such state is referred to as hibernating myocardium in which unlike myocardial stunning, the contractility is restored immediately once the blood flow is restored. The mechanisms responsible for the development of myocardial hibernation in which the heart reduces the contractile function in proportion to reduced blood flow are yet to be identified. The calcium responsiveness in experimental myocardial hibernation has been noted to be reduced and this reduction has not been related to decreased calcium sensitivity. Another important event of prolonged post ischemic reperfusion is no-reflow phenomenon in which no blood flow occurs through coronary blood vessels due to increased leukocyte-endothelial cell adhesion, platelet leukocyte aggregation, interstitial fluid accumulation and loss of endothelium dependent vasorelaxation, which all together result in mechanical blood flow obstruction (Maxwell and Lipp, 1997). The cellular and vascular effects due to prolonged ischemia are shown in **Fig. 2.**

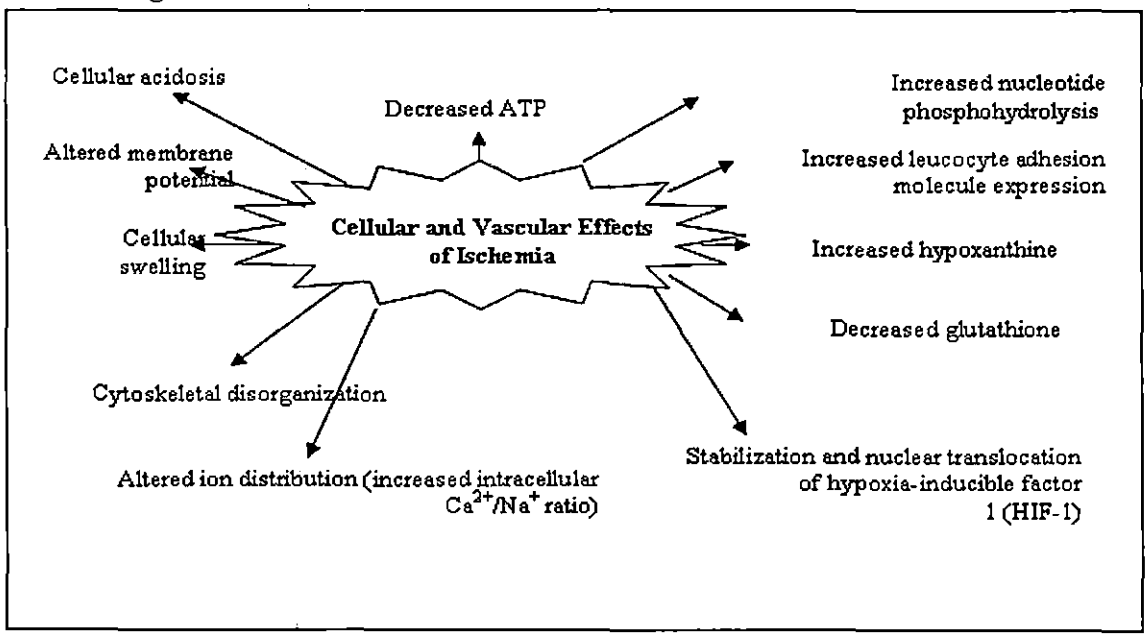

Fig.2.The cellular and vascular effects due to prolonged ischemia

Ischemia reduces cellular oxidative phosphorylation and thus the synthesis of energy rich phosphates is decreased, which alter the membrane ATP-dependent ionic pump function. This alteration favors the entry of calcium, sodium and water into cell which ultimately leads to cellular swelling. The reduced mitochondrial oxidative phosphorylation results in loss of major source of ATP production for energy metabolism. A compensatory increase in anaerobic glycolysis for ATP production leads to accumulation of hydrogen ions and lactate, resulting in intracellular acidosis (Buja, 2005). Moreover, ischemia promotes the expression of pro inflammatory genes, leukocyte adhesion molecules, endothelins and thromboxane A2 (Carden and Granger, 2000), which all together may affect the integrity of coronary vascular endothelium.

Polymorphonuclear leucocytes (PMNs) are mobilized from intravascular space to the interstitium during hypoxia and such responses may contribute significantly to tissue damage during subsequent reperfusion (Collard et al., 2002; Eltzschig *et al.,* 2003). The migration of PMNs through the endothelial barrier may disrupt such tissue barriers and create the potential for extra vascular fluid leakage and edema formation (Luscinskas *et al.,* 2002). The adenine nucleotide catabolism during ischemia leads to intracellular accumulation of hypoxanthine, which subsequently generates ROS upon reperfusion **Fig.3.**

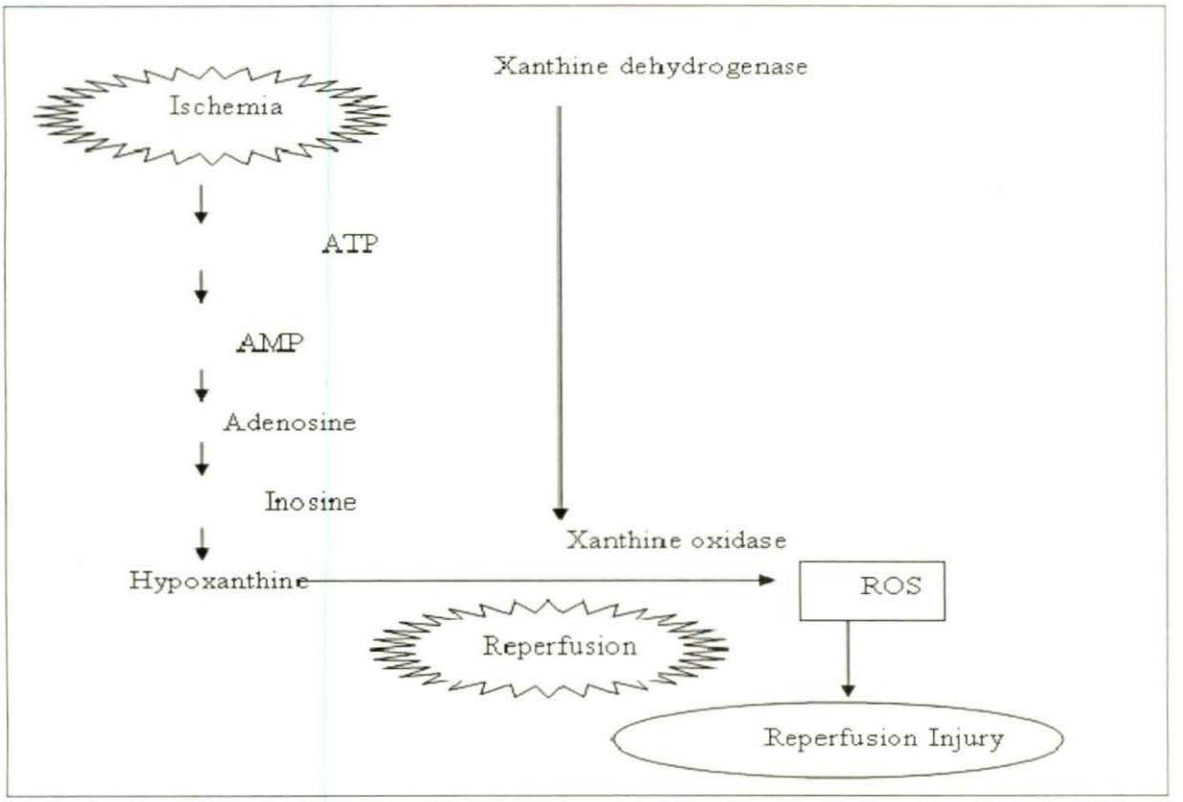

Fig.3. Formation of ROS in ischemia–reperfusion injury

During ischemia, cellular ATP is degraded to form hypoxanthine. Under normal condition, hypoxanthine is oxidized by xanthine dehydrogenase to xanthine, but during ischemia, xanthine dehydrogenase is converted to xanthine oxidase. Unlike xanthine dehydrogenase, which uses nicotinamide adenine dinucleotide as its substrate, xanthine oxidase uses oxygen and therefore, during ischemia, is unable to catalyze the conversion of hypoxanthine to xanthine, resulting in a buildup of excess tissue levels of hypoxanthine. When oxygen is reintroduced during reperfusion, conversion of the excess hypoxanthine by xanthine oxidase results in the formation of ROS (Charles et al., 2001) including superoxide anions (O_2-), hydroxyl radicals (OH-), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2) and peroxynitrite. ROS directly damage cellular membranes through lipid peroxidation. Further, ROS stimulate leukocyte activation and chemotaxis by activating plasma membrane phospholipase A2 to form arachidonic acid, an important precursor for synthesis of eicosanoids such as thromboxane A2 and leukotriene B4. Moreover, ROS stimulate leukocyte adhesion molecule and cytokine gene expression *via* activation of transcription

factors such as nuclear factor-kB (NF-kB). The multiple mechanisms have been postulated for the leukocyte-mediated tissue injury that occurs after ischemia/reperfusion. Micro vascular occlusion, increased vascular permeability (Bjork *et al.,* 1982) and release of oxygen free radicals (Fujita *et al.,* 1996), cytotoxic enzyme (Weiss, 1989) and inflammatory cytokines (Chaumoun *et al.,* 2000) have been demonstrated to contribute to leukocyte-induced tissue injury. I/R-induced leukocyte activation has been noted to release ROS, proteases and elastases, which result in increased micro vascular permeability, edema, and thrombosis and cell death.

Various signaling systems such as tumor necrosis factor- α (TNF-α, Rho-kinase, NF-κB, Janus Kinase (JAK), poly (ADP-ribose) polymerase (PARP), p38 mitogen activated protein kinase (MAPK), Caspases, interleukin-1 (IL-1) and IL-6 have been implicated in the pathophysiology of I/R injury. Further, polymorphonuclear leukocyte (PMN) and factor associated with neutral sphingomyelinase activation (FAN) have been noted to play a pivotal role in affected myocardium. Moreover, resident cardiac mast cells play a key role in I/R injury. The mast cells originate from pluripotent progenitor cells in bone marrow and are major players in the inflammation process. The degranulation of mast cells releases various cytotoxic mediators, which have been noted to be involved in the pathophysiology of ischemia/reperfusion injury.

2.3. PRECONDITIONING

In 1986, Murry and colleagues described an endogenous protective strategy in which multiple brief ischemic episodes in canine hearts limited infarct size from a subsequent sustained ischemic insult termed as ischemic preconditioning (IPC). The IPC has two phases of protection in which an early phase is lasting from few minutes to hours known as early preconditioning, and a late phase starts after 12 hours and lasts up to 3 days is referred to as delayed preconditioning (Yellon and Baxter, 1995; Bolli, 2000).

Following the finding of IPC by Murry and colleagues, several studies have investigated the mechanisms involved in its organ protective effects. By determining the mechanisms by which IPC confers myocardial preservation may eventually lead to the development of therapies to reduce cardiomyocyte injury following cardiopulmonary bypass. These studies led to the discovery that preconditioning could be induced by pharmacological means (Teoh

et al., 2002; Kevelaitis *et al.,* 2001) in which drugs are administered before the ischemic event with an intervening washout period before ischemia known as true pharmacological preconditioning or without a washout period known as pharmacological pretreatment.

The preconditioning may be triggered by substances like adenosine, bradykinin, NO, diazoxide, a mitochondrial ATP sensitive K^+ channel (KATP) opener, phospho kinase C (PKC) activators, opioids and prostaglandins (Post and Heusch, 2002). In addition, anesthetics were investigated for their potential to precondition the heart before ischemia. All halogenated, volatile substances were found to be protective and their actions were comparable to that of ischemic preconditioning (Chiari *et al.,* 2005; Przyklenk *et al.,* 2003)

Short periods of ischemia in remote vessels or even distant organs protected the myocardium from injury induced by coronary artery reperfusion. Thus, the substances must have been released from the remote ischemic reperfused tissue that protected the jeopardized myocardium. The occlusion of circumflex artery has produced the protection of myocardium supplied by left anterior descending coronary artery and this phenomenon is termed as intra cardiac preconditioning (Przyklenk *et al.,* 1993). The short occlusions of renal artery (Mclahan *et al.,* 1993; Pell *et al.,* 1998) abdominal aorta (Weinbrenner *et al.,* 2002; Singh *et al.,* 2004) and mesenteric artery have been documented to protect myocardium against I/R-induced injury. This phenomenon is termed as remote preconditioning (Heusch and Schulz, 2002; Wang *et al.,* 2001) or intra organ preconditioning or preconditioning at a distant site (Schoemaker, 2000).

2.4. POSTCONDITIONING

The brief intermittent episodes of ischemia and reperfusion, at the onset of reperfusion after a prolonged period of ischemia confer cardio protection; a phenomenon is termed as ischemic post conditioning. This concept was first introduced by Zhao *et al.,* 2006. In a canine left anterior descending coronary artery ligation (LAD) model, they compared the protective effects of IPC to that of post conditioning. The brief ischemia and reperfusion of 30 seconds each after prolonged ischemia significantly reduced infarct size and endothelial dysfunction (Zhao *et al.,* 2006). The word post conditioning was given since the stimulus (10-30 seconds for 3-6 times) is applied after a period of ischemia. It has been proposed that passive and active phases are involved in cardio protective mechanisms of post-

conditioning. The passive phase is initiated via stepwise reperfusion that reduces the delivery of oxygen radicals and mitochondrial Ca^{2+} overload.

In active phase, the reperfusion injury salvage kinases (RISK) pathways which include phosphatidylinositol-3-OH kinase (PI3K), akt(protein-Ser/Thr kinase) and extracellular signal-regulated kinase (ERK-1/2) are activated by endogenous stimulators such as adenosine, opioids and some unidentified endogenous substances (Tsang *et al.,* 2004; Yang *et al.,* 2004; Morrison *et al.,* 2007). Post-conditioning mediated activation of PI3 kinase, Akt and subsequently endothelial NO synthase (eNOS) inhibit the opening of mitochondrial permeability transition pore (mPTP) to afford cardio protection (**Fig. 4.**) (Gross, 2006).

Further, post conditioning activates p70s6K through mitogen-activated protein kinase (MEK ½) and ERK ½ signaling systems that initiates protein translation to mediate cardio protection. It has been suggested that post conditioning mediated cardio protection is likely produced via the ERK1/2 pathway rather than PI3 kinase/Akt pathways. On the other hand, it has been noted that Akt and ERK activated during post conditioning do not protect myocardium from reperfusion injury (Schwartz and Lagranha, 2006).

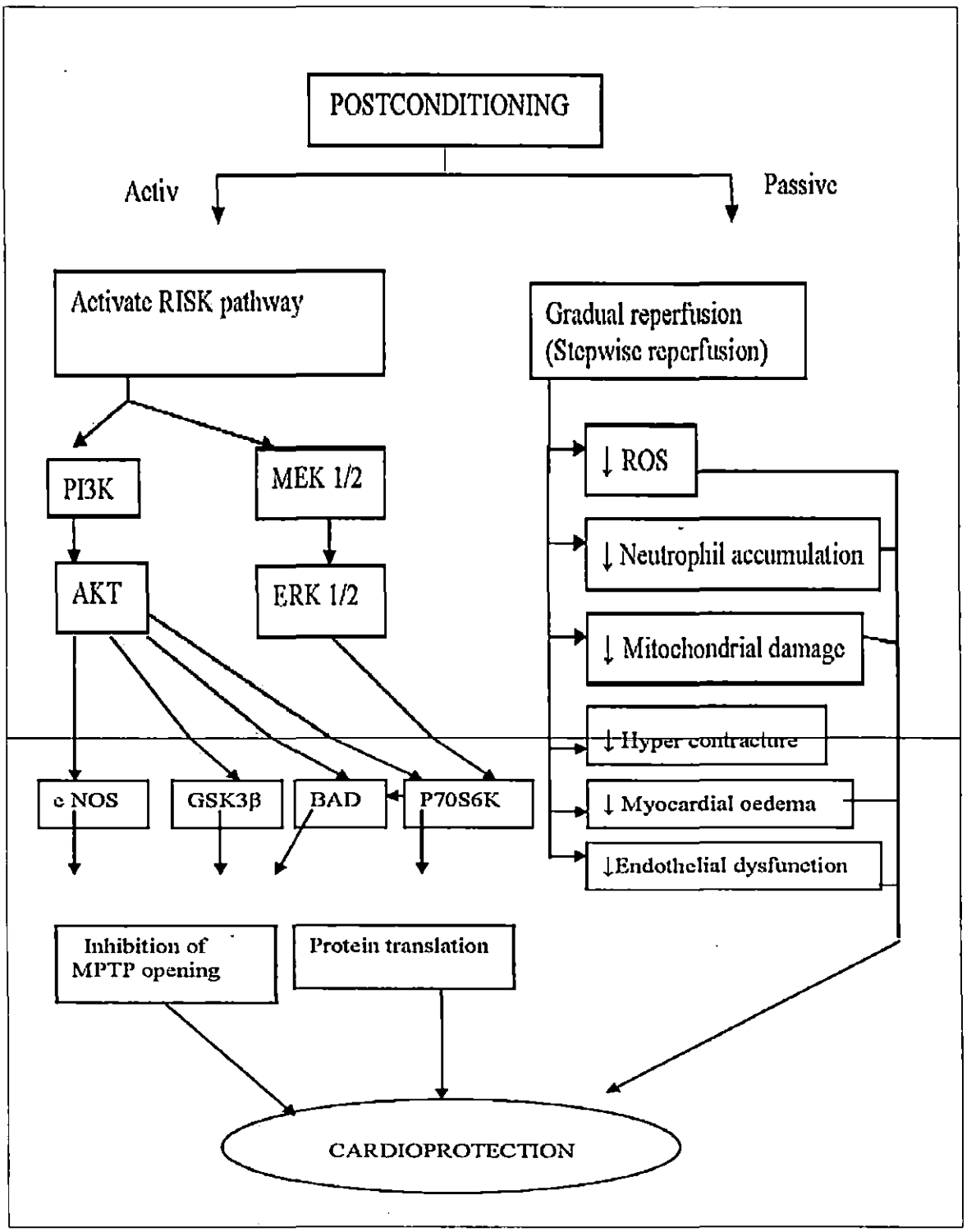

Fig.4. Post conditioning mediated cardio protection

2.5. CLINICAL RELEVANCE OF PRECONDITIONING AND POST CONDITIONING

Numerous *in vitro* findings suggest that the human myocardium can be protected by ischemic preconditioning. Ischemia required for the insertion of coronary artery bypass grafts during cardiac surgery has been shown to provide cardio protection (Jenkins *et al.*, 1995). The findings from many preclinical studies in which cardio protection has been seen in healthy animal hearts might not be reproducible in the human myocardium since human ischemic heart disease is frequently associated with various disorders such as diabetes mellitus and its complications and left ventricular hypertrophy etc or with other contributing factors like older age. The presence of these conditions might interrupt with the protection induced by ischemic preconditioning (Schulman *et al.*, 2001; Tsang *et al.*, 2005). The use of pharmacologic agents to target different components of the signaling pathway that mimic the protection induced by ischemic preconditioning known as pharmacologic preconditioning, might enable this approach to be recognized as a clinical therapy (Ramzy *et al.*,2007; Vinten *et al.*, 2007). Nicorandil has cardio protective effects when given as an adjunctive therapy at the time of reperfusion in cardiac patients during surgery (Ono *et al.*, 2004; Kloner *et al.*, 2007).

Preclinical studies demonstrated that pharmacologic inhibition of Na^+/H^+ exchanger before myocardial ischemia could reduce infarct size through a reduction in myocardial calcium accumulation, to a level comparable to ischemic preconditioning (Avkiran and Marber, 2002; Yellon and Hausenloy 2005). Adenosine has been shown to be a great promising cardio protective agent in different clinical settings of myocardial I/R (Quintana *et al.*, 2004). The post conditioning has been shown to be effective in patients with coronary artery disease. Marked improvement in coronary blood flow has been noted in post conditioned patients (Statt *et al.*, 2005). Taken together, post conditioning would be a safe cardio protective intervention to reduce reperfusion injury in patients with ischemic heart diseases.

2.6. MOLECULAR AND CELLULAR MECHANISMS OF MYOCARDIAL ISCHEMIA REPERFUSION INJURY

Reperfusion injury manifestations include reperfusion arrhythmias, endothelial cell damage leading to micro vascular dysfunction, myocardial stunning, myocyte death and infarction. Normal cardiac function is predicated on a continuous supply of oxygen and nutritive substances. When coronary perfusion is interrupted, profound myocardial damage can occur at both microscopic and macroscopic levels. Clinically, this scenario gives rise to acute coronary syndromes manifesting as angina, or in the most severe form, acute myocardial infarction. The onset of ischemia triggers homeostatic processes geared at limiting damage, but which may act in concert with processes associated with reperfusion, to actually exacerbate injury.

As a result of intensive investigation over decades, a detailed understanding is now available of the complexity of the response of the myocardium to an ischemic insult. Myocardial ischemia results in a characteristic pattern of metabolic and ultra structural changes that lead to irreversible injury. Recent studies have explored the relationship of myocardial ischemic injury to the major modes of cell death, namely, oncosis and apoptosis. The evidence indicates that apoptotic and oncotic mechanisms can proceed together in ischemic myocytes with oncotic mechanisms and morphology dominating the end stage of irreversible injury. Reperfusion of ischemic myocardium leads to severe damage, which is indicated by free radicals, intracellular calcium overloading and loss of membrane phospholipids (Maxwell and Lip, 1997; Dobsak et al., 2003). Malondialdehyde (MDA), a stable metabolite of the free radical-mediated lipid peroxidation cascade, is widely used as marker of oxidative stress. Glutathione (GSH) is an important endogenous antioxidant the levels of which are influenced by oxidative stress.

Myocardial infarcts evolve as a wave front of necrosis, extending from sub endocardium to sub epicardium over a 3 to 4 hour period. A number of processes can profoundly influence the evolution of myocardial ischemic injury. Timely reperfusion produces major effects on ischemic myocardium, including a component of reperfusion injury and a greater amount of salvage of myocardium.. Preconditioning by several short bouts of coronary occlusion and reperfusion can temporarily salvage significant amounts of myocardium and extend the

window of myocardial viability. Ongoing research into the mechanisms involved in reperfusion and preconditioning is yielding new insights into basic myocardial pathobiology.

2.7. ENDOTHELIAL FUNCTION IN CARDIOVASCULAR DISEASES

The vascular endothelium is involved in the release of various vasodilators, including NO, prostaglandins and endothelium-derived hyperpolarizing factor as well as vasoconstrictors. The NO plays an important role in the regulation of vascular tone, inhibition of platelet aggregation and suppression of smooth muscle cell proliferation. Impaired endothelium-dependent vasodilation has been found in the forearm, coronary, and renal vasculature in patients with cardiovascular diseases. Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis. In patients with coronary artery diseases, (Suwaidi *et al.,* 2000), found that severe coronary endothelial dysfunction is associated with increased cardiovascular events. Schachinger *et al.,* 2000 demonstrated a link between coronary endothelial dysfunction and subsequent cardiovascular events in patients with coronary artery disease. These clinical studies have shown that endothelial function can serve as an independent predictor of cardiovascular events. From a clinical perspective, it is important to select an appropriate intervention that is effective in improving endothelial function in patients with cardiovascular diseases. Several investigators (Ress DD *et. al.*, 1989, Cai H *et. al.*, 2000 Delles C *et. al.*, 2002) have reported possible mechanisms of impairment of endothelial function in cardiovascular diseases; abnormalities of shear stress, increase in the amount of endogenous endothelial NO synthase (eNOS) inhibitor, asymmetrical dimethylarginine, increases in the amount of vasoconstrictors, such as angiotensin II (Ang II), endothelin-1, and nor-epinephrine, and inactivation of NO by ROS. The growing evidence has shown an interaction between oxidative stress and endothelial function. Enhanced production of ROS and an attenuated antioxidant system may contribute to endothelial dysfunction in cardiovascular diseases. In other words, enhanced NO inactivation caused by excess ROS production, rather than decreased NO production, may play an important role in the impaired endothelium-dependent vasodilation in cardiovascular diseases. These findings suggest that a decrease in NO inactivation contributes to the improvement in endothelial function in patients with cardiovascular diseases.

2.8. MECHANISMS OF ISCHEMIC INJURY

Early observations on the mechanisms of ischemic injury focused on relatively simple biochemical and physiological changes which were known to result from interruption of circulation. Examples of these changes are loss of high-energy compounds, acidosis due to anaerobic generation of lactate. Subsequent research has shown the problem to be far more complex than was previously thought, involving the action and interaction of many factors which are discussed as follows.

2.8.1. Free Radicals

The radicals which have unpaired electrons and potent ability of oxidation are called as free radicals. The sources and metabolism of reactive oxygen species (ROS) are as follows.

ROS includes superoxide anion (O^{2-}.), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), hypochlorous acid (HOCl), nitric oxide (NO), and peroxynitrite (ONOO-). Amongst these O^{2-}, OH and NO are classified as free radicals whereas H_2O_2, HOCl, and ONOO- are classified as non-free radicals that also have the ability to oxidize. The sources of ROS are a variety of cell types such as vascular smooth muscle cells, endothelial cells, and mononuclear cells. Potential sources of ROS production include nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, NO synthase, mitochondrial electron transport, cyclooxygenase, glucose oxidase, and lipooxygenase (**Fig. 5**). These various oxidase enzymes produce superoxide. The antioxidant enzyme superoxide dismutase (SOD) rapidly dismutates superoxide to H_2O_2. Then H_2O_2 is eliminated by glutathione peroxidase (GPX) and catalase to water.

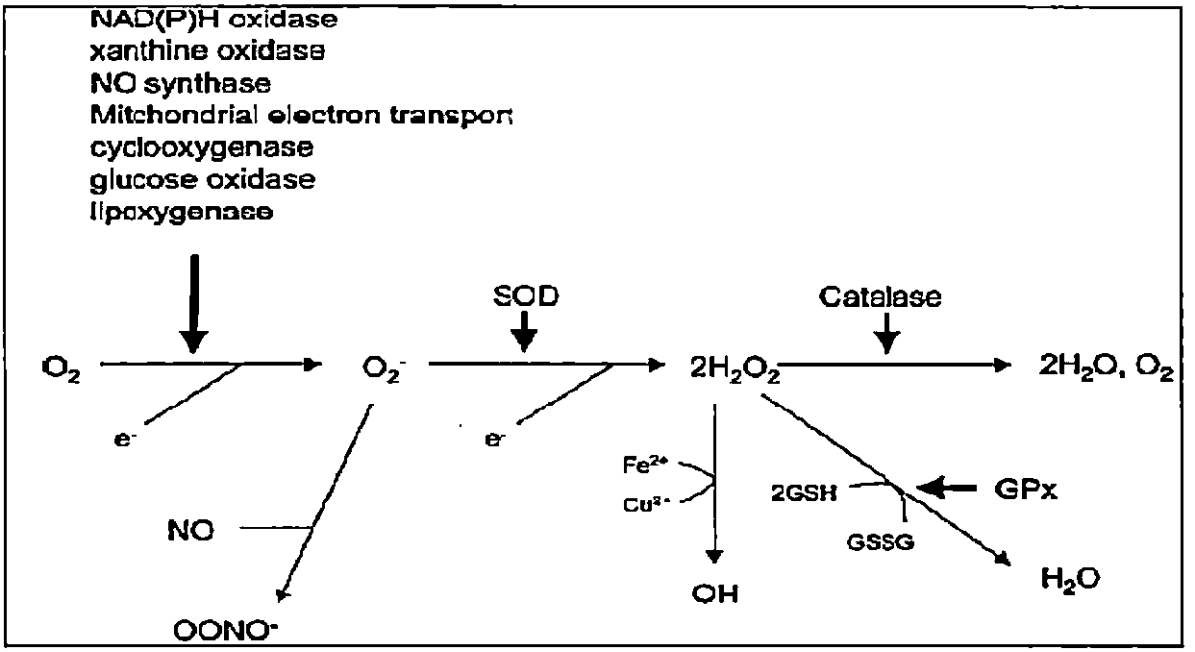

Fig.5. Metabolism of reactive oxygen species.

NADPH indicates nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; O_2, oxygen; O^{2-}, superoxide anion; e, electron; SOD, superoxide dismutase; H_2O_2, hydrogen peroxide; H_2O, water; OONO-, peroxynitrite; OH, hydroxyl radical; GSH,glutathione; GSSG, disulfide of glutathione ,GPX, glutathione peroxidase.

During ischemia, the hydrolysis of ATP via AMP leads to an accumulation of hypoxanthine. The increased intracellular calcium enhances the conversion of xanthine dehydrogenase (XD) to xanthine oxidase (XO). Upon reperfusion and reintroduction of oxygen, XO may produce superoxide and xanthine from hypoxanthine and oxygen. Even more damaging free radicals could conceivably be produced by the metal catalyzed Haber-Weiss reaction as follows:

O_2- + H_2O ----Fe^{3+} ------> O_2 + OH-+ OH-

Iron, the transition metal needed to drive this reaction, is present in abundant quantities in bound form in living systems in the form of cytochromes, transferrin and hemoglobin. Anaerobic conditions have long been known to release such normally bound iron. Indirect experimental confirmation of the role of free iron in generating free-radical injury has come from a number of studies which have confirmed the presence of free-radical breakdown products such as conjugated dienes and low molecular weight species of iron.

During reperfusion and re-oxygenation, significantly increased levels of several free-radical species that degrade cell and capillary membranes have been postulated: O2-, OH-, and free lipid radicals (FLRs). The super oxide ion may be formed by the previously described actions of XO and/or by release from neutrophils which have been activated by leukotrienes. The re-oxygenation also restores ATP levels and this may in turn allow active uptake of calcium by the mitochondria, resulting in massive calcium overload and destruction of the mitochondria.

2.8.2. Calcium

Normally, calcium is present in the extracellular milieu at a concentration of 10,000 times greater than the intracellular concentration. This 10,000:1 differential is maintained by at least the following four mechanisms:

i. Active extrusion of calcium from the cell by an ATP-driven membrane pump.

ii. Exchange of calcium for sodium at the cell membrane driven by the intracellular to extracellular differential in the concentration of Na+ as a result of the cell membrane's Na^+ - K^+ pump.

iii. Sequestration of intracellular calcium in the endoplasmic reticulum by an ATP-driven process.

iv. Accumulation of intracellular calcium by oxidation-dependent calcium sequestration inside the mitochondria.

The loss of cellular high-energy compounds during ischemia causing the loss of the Na^+ -K^+ gradient, virtually eliminates three of the four mechanisms of cellular calcium homeostasis. This, in turn, causes a massive and rapid influx of calcium into the cell. Mitochondrial sequestration, the remaining mechanism, causes overloading of the mitochondria with calcium and diminished capacity for oxidative phosphorylation. Elevated intracellular Ca^{++} activates membrane phospholipases and protein kinases. A consequence of phospholipase activation is the production of free fatty acids (FFA's) including the potent prostaglandin inducer, arachidonic acid (AA). The degradation of the membrane by phospholipases almost certainly damages membrane integrity, further reducing the efficiency of calcium pumping and leading to further calcium overload and a failure to regulate intracellular calcium levels

following the ischemic episode. Additionally, FFAs almost certainly have other degradative effects on cell membranes. The production of AA as a result of FFA release causes a biochemical cascade ending with the production of thromboxane and leukotrienes. Both these compounds are profound tissue irritants which can cause platelet aggregation, clotting, vasospasm, and edema, with resultant further compromise to restoration of adequate cerebral perfusion upon restoration of blood flow.

2.8.3. Mitochondrial Dysfunction

Calcium loading and free-radical generation are no doubt major contributors to the mitochondrial ultra structural changes which are known to occur following cerebral ischemia. In addition to the structural alterations observed, there are biochemical derangements such as a marked decrease in adenine nucleotide translocase and oxidative phosphorylation. There is also an accumulation of FFAs, longchain acylCoA, and long-chain carnitines. Of these alterations, the accumulation of long-chain acylCoA is perhaps most significant, since intramitochondrial accumulation of long chain acylCoA is known to be deleterious to many different mitochondrial enzyme systems.

2.8.4. Lactic Acidosis

Lactic acidosis does apparently contribute to the pathophysiology of ischemia while it is clearly not the sole or even the major source of injury in ischemia. It has been shown, for instance, that lactate levels above a threshold of 18 - 25 micromol/g result in currently irreversible injury. Decrease in pH as a consequence of lactic acidosis has been shown to injure and inactivate mitochondria. Lactic acid degradation of NADH (which is needed for ATP synthesis) may also interfere with adequate recovery of ATP levels post ischemically. Lactic acid can also increase iron de compartmentalization, thus increasing the amount of free-radical mediated injury.

2.8.5. Neutrophil Activation

Since the late 1960s, polymorphonuclear leukocytes (PMNLs) and monocytes/ macrophages have been implicated as significant causes of pathology in ischemia. During the last decade there has been a veritable explosion of research documenting the role of PMNLs in reperfusion injury. Most of the initial work done in this area focused on PMNL-mediated

reperfusion injury to the myocardium, establishing that PMNL activation and subsequent plugging and degranulation (resulting in release of oxidizing compounds) is responsible for the no-reflow phenomenon following myocardial ischemia. In particular, the work of Engler has demonstrated that PMNL activation is responsible for plugging at least 27% of myocardial capillaries and is further responsible for the development of edema and arrhythmias upon reperfusion

To what extent leukocyte plugging occurs in the brain following global ischemia remains controversial. Recent study answered the question of how rapidly leukocyte plugging occurs following ischemia. It was noted that no leukocyte plugging after 3 hours of reperfusion following a 40-minute ischemic episode. However, it is clear from a growing body of work that neutrophils are a major mediator of ischemic injury in a variety of organ systems and that their acute activation is responsible for many of the effects of ischemia observed in the brain and other body tissues, including the loss of capillary integrity and the degradation of ultra structure upon reperfusion.

2.8.6. TNF-α

Recent studies have focused their attention on the role of the proinflammatory cytokine tumor necrosis factor (TNF) in the development of heart failure. In addition, both in vivo and in vitro studies demonstrate that TNF effects cellular and biochemical changes that match those seen in patients with congestive heart failure. Furthermore, in animal models, the development of the heart failure phenotype can be abrogated at least in part by anti cytokine therapy. Based on information from experimental studies, investigators are now evaluating the clinical efficacy of novel anti cytokine and anti-TNF strategies in patients with heart failure.

Healing of myocardial infarcts depends on an inflammatory cascade that ultimately results in clearance of dead cells and matrix debris and formation of a scar. Myocardial necrosis activates complement, Nuclear Factor (NF)-κB and Toll-like Receptor (TLR)-dependent pathways, and generates free radicals, triggering an inflammatory response. Chemokines and cytokines are markedly induced in the infarct and mediate recruitment and activation of neutrophils and mononuclear cells. Extravasation of platelets embedded in a mesh of cross linked fibrin. This provisional matrix provides a scaffold for migration of cells into the

infarct. Monocytes differentiate into macrophages and secrete fibrogenic and angiogenic growth factors inducing formation of granulation tissue, containing myofibroblasts and neovessels. Repression of proinflammatory cytokine and chemokine synthesis, mediated in part through Transforming Growth Factor (TGF)-β and Interleukin (IL)-10, is critical for resolution of the inflammatory infiltrate and transition to fibrous tissue deposition. Infarct myofibroblasts deposit extracellular matrix proteins and a collagen-based scar is formed. As the wound matures, fibroblasts undergo apoptosis and neovessels regress, resulting in formation of a scar with a low cellular content containing dense, cross-linked collagen. The pathologic and structural changes associated with infarct healing directly influence ventricular remodeling and affect prognosis in patients with myocardial infarction. Understanding the mechanism involved in the regulation of the post-infarction inflammatory response, and the spatial and temporal parameters of wound healing is necessary in order to identify specific molecular targets for therapeutic intervention.

2.8.7. Opportunities for Intervention

i. Numerous studies have suggested a cardio protective effect for a variety of calcium channel blockers administered post-insult.

ii. Free radical damage: Free radicals have long been understood to be a major source of ischemic pathology. Similarly, there have been a number of studies which suggest that free radical associated ischemic injury can be reduced greatly or eliminated by pre or post insult treatment with nutritional antioxidants such as vitamin E, selenium, vitamin C, and beta carotene. Theoretical considerations also suggested other possible therapeutic agents such as those known to elevate neuronal (intracellular) glutathione levels for protection from ischemic injury.

iii. Phospholipase activation has been implicated as a significant source of injury in both cold and warm ischemia. The phospholipase inhibitor quinacrine has reduced cold ischemic injury in an organ preservation model as well as myocardial reperfusion injury.

iv. Inhibition of the inflammatory cascade and the adhesion and degranulation of polymorphonuclear lymphocytes by both drug treatment and by their removal via filtration have been shown to lessen reperfusion injury in the heart.

2.9. INTERNATIONAL STATUS ON CVD

CVD is a leading cause of mortality and is responsible for one third of global death. The majority of the estimated 32 million heart attacks strokes that occur every year are caused by one or more CVD risk factors such as hypertension, diabetes smoking, high levels of blood lipid and physical inactivity. In China, the corresponding figure is 35% an estimated 16.7 million or 29.2% of total global death result from the various form of cardiovascular disease. More than 50% of death and disability from heart disease and strokes which together kill more than 12 million people per year. An estimated 16.7 million of 29.2% of total global deaths result from the various forms of cardiovascular disease (CVD).

Cardiovascular disease (CVD) has been the dominant cause of death in Australia for many decades, with coronary heart disease (CHD) and stroke ranking high among leading causes of death. CVD accounts for more than 46,000 deaths annually in Australia, and 3.7 million Australians have a long-term cardiovascular disease (AIHW 2008). There is some evidence from the United Kingdom and the United States that CHD mortality rate declines have accelerated in some older age groups but slowed in some younger age groups in the recent past (Allender *et al.*, 2008, Ford & Capewell 2007 and O'Flaherty *et al.*, 2008). An estimated 17.5 million people died from cardiovascular disease in 2005, representing 30% of all global deaths. Of these deaths, 7.6 million were due to heart attacks and 5.7 million due to stroke. About 80% of these death occurred in low and middle income countries. If current trends are allowed to continue, by 2015 an estimated 20 million people will die from cardiovascular disease (WHO, 2005)

2.10. NATIONAL STATUS ON CVD

CVD is the number one cause of mortality in India today. Previously, CVD was considered to be a result of an urban lifestyle; however, many recently published studies have indicated that CVD is also on the rise in rural areas. (Patil *et al.*, 2004; Goel *et al.*, 2003) The prevalence of CVD is four-fold higher in urban India and two-fold higher in rural India than

in the United States (Goel *et al.,* 2003). Apart from current lifestyle factors, there is an additional 3–4-fold risk from genetic and ethnic factors for heart disease. Certain ethnic groups in India, like the Jains, Marwaris, Banias and other communities belonging to the states of Rajasthan, Gujarat, Haryana and Punjab, are genetically predisposed to central obesity. (Dwivedi, 2004) They are at a higher risk of developing CVD. The picture is even gloomier for migrant Indians. (Hoogeveen *et al.,* 2001)

A new research publication in *The Lancet 2008* finds that India will bear 60% of the world's heart disease burden in the next two years. In addition, researchers have determined that compared to people in other developed countries, the average age of patients with heart disease is lower among Indian people and Indians are more likely to have types of heart disease that lead to worse outcomes. The leading cause of death in the world is ischemic heart disease, a condition characterized by reduced blood supply to the heart that is usually due to coronary artery disease. In 2001 alone, some 7.1 million deaths were attributed to ischemic heart disease, 80% of which were in relatively poor countries. Medical and public health professionals expect that in developing countries, there will be a 137% and 120% increase in the disease for males and females, respectively, whereas these predictions lie in the 30% to 60% range for developed countries.

As per WHO report the morbidity and mortality due to cardiovascular diseases in India will reach an epidemic proportion by the middle of this century. In India, approximately 53% of CVD deaths are in people younger than 70 years of age. The prevalence of CAD in urban North India varies from 7% to 10% compared to 3% in USA and <1% in Japan. The CAD rates in South India are two folds higher than in North India, with Kerala reporting 14% in urban and 7% in rural Cardiovascular diseases particularly IHD, have become a worldwide health problem affecting all economic groups of the society and are responsible for 35% of all deaths in the world. The mortality due to CVD ranges from 16% to 50% in the developing and developed countries. Since 78% of all deaths of the world occur in the developing countries, 85% of the global death and disease burden from CVD is borne by low and middle income countries. In India app 53% of CVD deaths are in people younger than 70years of age. By 2020 CVD will be the leading cause of death in developing countries like India.

2.11. PHARMACOLOGY OF PYRAZOLONES

The various pharmacological aspects of pyrazolone derivatives and its role on various mediators which are associated with CVS are described as follows.

2.11.1. Cardioprotective activity

Edaravone (3-methyl-1-phenyl-2-pyrazolone), a strong free radical scavenger **(Fig.6.)** is used for treatment of patients with acute brain infarction. Edaravone has been developed by Mitsubishi-Tokyo Pharmaceuticals Inc (Tokyo, Japan). Edaravone has preventive effects on myocardial injury following ischemia and reperfusion in patients with acute myocardial infarction. Antioxidant actions of edaravone include enhancement of prostacyclin production, inhibition of lipoxygenase metabolism of arachidonic acid by trapping hydroxyl radicals, inhibition of alloxan-induced lipid peroxidation, and quenching of active oxygen, leading to protection of various cells, such as endothelial cells, against damage by reactive oxygen species (ROS). The novel free radical scavenger edaravone may represent a new therapeutic intervention for endothelial dysfunction in the setting of atherosclerosis, chronic heart failure, diabetes mellitus, or hypertension. In addition, edaravone improves endothelial function in smokers through an increase in nitric oxide (NO) bioavailability.

This focused on clinical findings and on putative mechanisms underlying the beneficial effects of the antioxidative agent edaravone on the atherosclerotic process in patients with cardiovascular diseases.

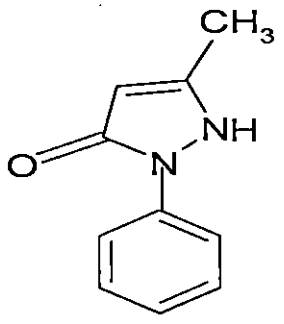

Fig.6. Structure of 3-methyl-1-phenyl-2-pyrazolone (Edarvone)

2.11.2. Benefits of edarvone (pyrazolone) for cardiovascular diseases

It is expected that edaravone has beneficial effects on coronary artery and myocardial cells after ischemic and post-ischemic myocardial injury in patients with ischemic heart diseases, including acute myocardial infarction and angina pectoris. Some animal studies using acute myocardial ischemia-reperfusion models have suggested protective effects of edaravone on myocardial damage. Yanag isawa *et al.,* 1994 showed that intravenous infusion of edaravone at a dose of 3 mg/kg attenuates the loss of myocardial creatine kinase activity from the left ventricular free wall in rats subjected to coronary artery occlusion for 10 minutes followed by reperfusion for 24 hours and reduced infarct size by approximately 50% compared with that in the control vehicle group. Minhaz *et al.,* 1996 reported that edaravone attenuated the myocardial necrotic area by approximately 50% in isolated reperfusion rat heart subjected to coronary artery occlusion.

This beneficial effect was related to reduction in myocardial damage. Also, in rabbit hearts subjected to ischemic reperfusion, a bolus infusion of edaravone reduced the necrotic area (Wu *et al.,* 2000). It has been reported that edaravone at a dose of 15 mmol reduced the death of isolated adult rabbit ventricular cells by approximately 40% compared with that in the control vehicle group (Yamawaki *et al.,* 2004).

Tsujita *et al.,* 2004 investigated the effects of edaravone on left ventricular function and infarct size using a randomized, placebo-controlled, open-label protocol in 80 patients with acute myocardial infarction. Intravenous administration of edaravone at a dose of 30 mg for 10 minutes before myocardial reperfusion decreased serum concentrations of creatine kinase-MB isoenzymes, a surrogate point of infarct size, and improved left ventricular ejection fraction in patients with acute myocardial infarction compared with those in the placebo group (**Fig.7**).

These findings suggested that edaravone has cardioprotective effects. This drug had shown protective effect on post ischemic injury in the coronary vasculature and myocardium in patients with cardiovascular diseases though a decrease in oxidative stress. In other post ischemic reperfusion models, the usefulness of edaravone for organ protection has been reported.

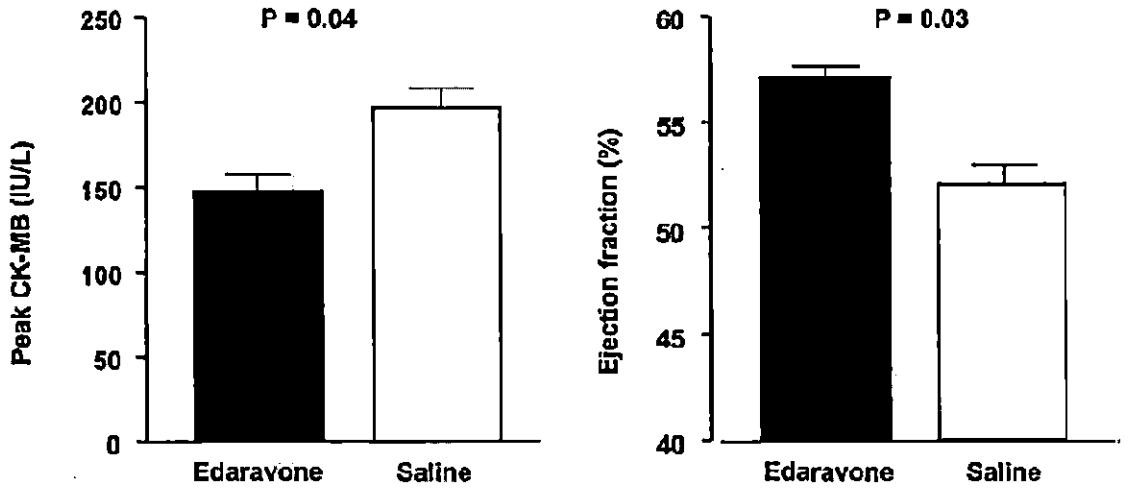

Fig.7. Effects of edaravone on peak CK-MB and ejection fraction in patients with acute myocardial infarction

Edaravone improves gastrocnemius and tibialis anterior muscles injury in a rat ischemic limb model and prevents kidney post ischemic reperfusion injury in rats(Masaki *et al.,* 1996) and lipopolysaccharide-induced liver damage in rats (Kono *et al.,* 2003) . These findings suggest that edaravone may have beneficial effects on ischemia-reperfusion injury in various muscles, vessels, and tissues in different organs.

2.11.3 Putative mechanisms underlying antioxidant action of edaravone

After ischemia-reperfusion, large amounts of ROS are produced from vascular smooth muscle cells, endothelial cells, and mononuclear cells. It has been shown that edaravone reduces or restores the amount of ROS increased by post ischemic reperfusion and prevents impairment of the antioxidant defense system (Tosaka *et al.,* 2002; Yamaguchi *et al.,* 2003). Scavenging ROS by edaravone may play a key role in preventing post ischemic reperfusion injury in various types of cells and tissues. The reported antioxidant actions of edaravone include

i. Enhancement of prostacyclin production,
ii. Inhibition of lipoxygenase metabolism of arachidonic acid by trapping hydroxyl radicals

iii. Inhibition of alloxan-induced lipid peroxidation,

iv. Quenching of reactive oxygen,leading to protection of various cells, such as endothelial cells, against damage by ROS (Kawai *et al.,* 1997).

The putative mechanism underlying the antioxidant action of edaravone is as follows (Yamamoto *et al.,* 1996) **(Fig.8.)**: an electron transfer from an edaravone anion to peroxyl radical yields an edaravone radical and peroxyl anion, and this reaction breaks the chain oxidation of lipids. Then, edaravone peroxyl radical transforms to 4, 5-dione by elimination of a hydrogen atom and one electron. Finally, 2-oxo-3-(phenylhydrazono)- butanoic acid (OBP) is produced by the hydrolysis of 4,5- dione.

It is thought that edaravone exists near the cell membrane or perhaps on the cell membrane. Edaravone has a low molecular weight (MW 174.2), is both lipid-soluble and water-soluble, and has good cell membrane permeability (Yamamoto *et al.,* 1996). It has been confirmed that edaravone has the ability to pass through the blood-brain barrier in dogs. Edaravone directly prevents hydroxyradical-induced injury of cultured bovine aortic endothelial cells. In addition, edaravone stimulates the conversion of arachidonic acid to prostacyclin and inactivates ROS, resulting in protection of endothelial cells.

Interestingly, edaravone induced endothelial NO synthase (eNOS) in the ischemic spinal cord in rabbits, preventing spinal cord damage (Takahashi *et al.,* 2004), and it also restored the reduced expression of eNOS, mRNA and protein in the rabbit artery following irradiation (Zhang *et al.,* 2003).

Yoshida et al 2005 recently reported that edaravone enhances the expression of eNOS and restores the reduction in eNOS by oxidized low-density lipoprotein in endothelial cells. These findings suggest that edaravone prevents the cell damage induced by oxidative stress through not only direct ROS scavenging effect but also restoration of reduced eNOS expression.

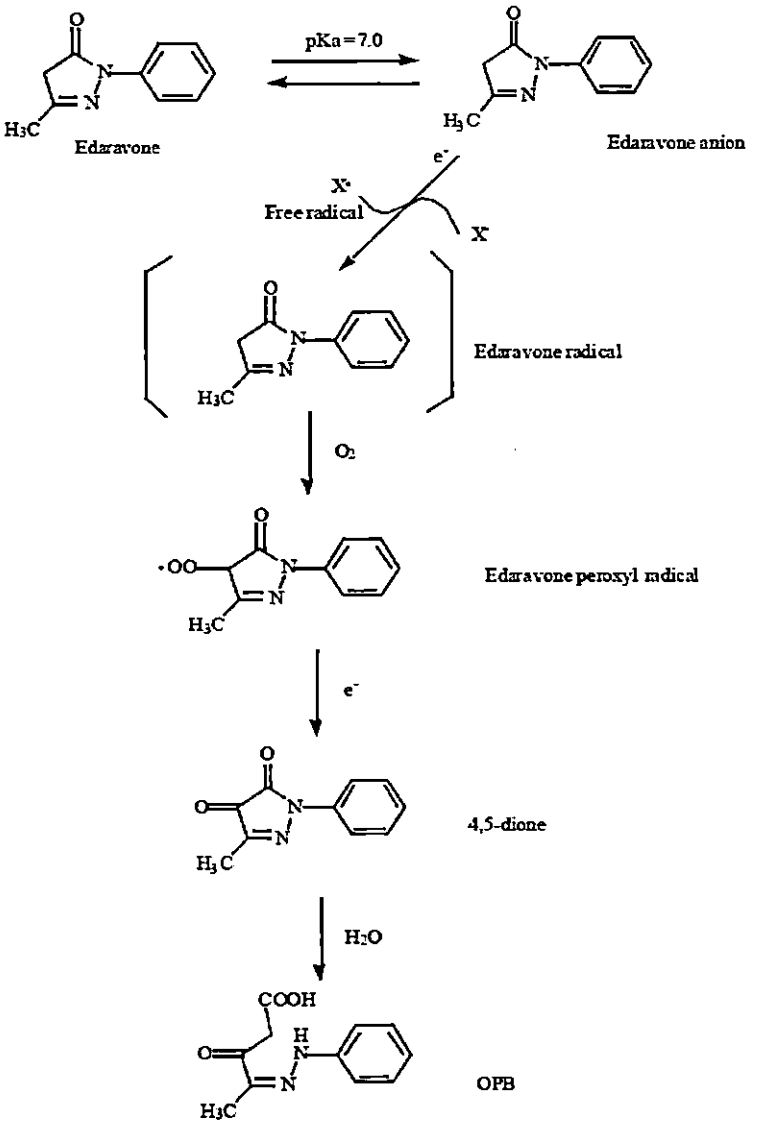

Fig.8. Putative mechanisms of antioxidant actions of edaravone. OPB indicates 2-oxo-3-(phenylhydrazono)-butanoic acid. (Hirokawa, T., Sugawara, K., Tanaka, T.: Japan Patent 04091441A2 (2004)

The novel free radical scavenger edaravone may represent a new therapeutic intervention for endothelial dysfunction in the setting of atherosclerosis, chronic heart failure, diabetes mellitus, or hypertension through its free radical scavenging and antioxidant actions. The role of edaravone in CVD is shown in the **Fig.9.**

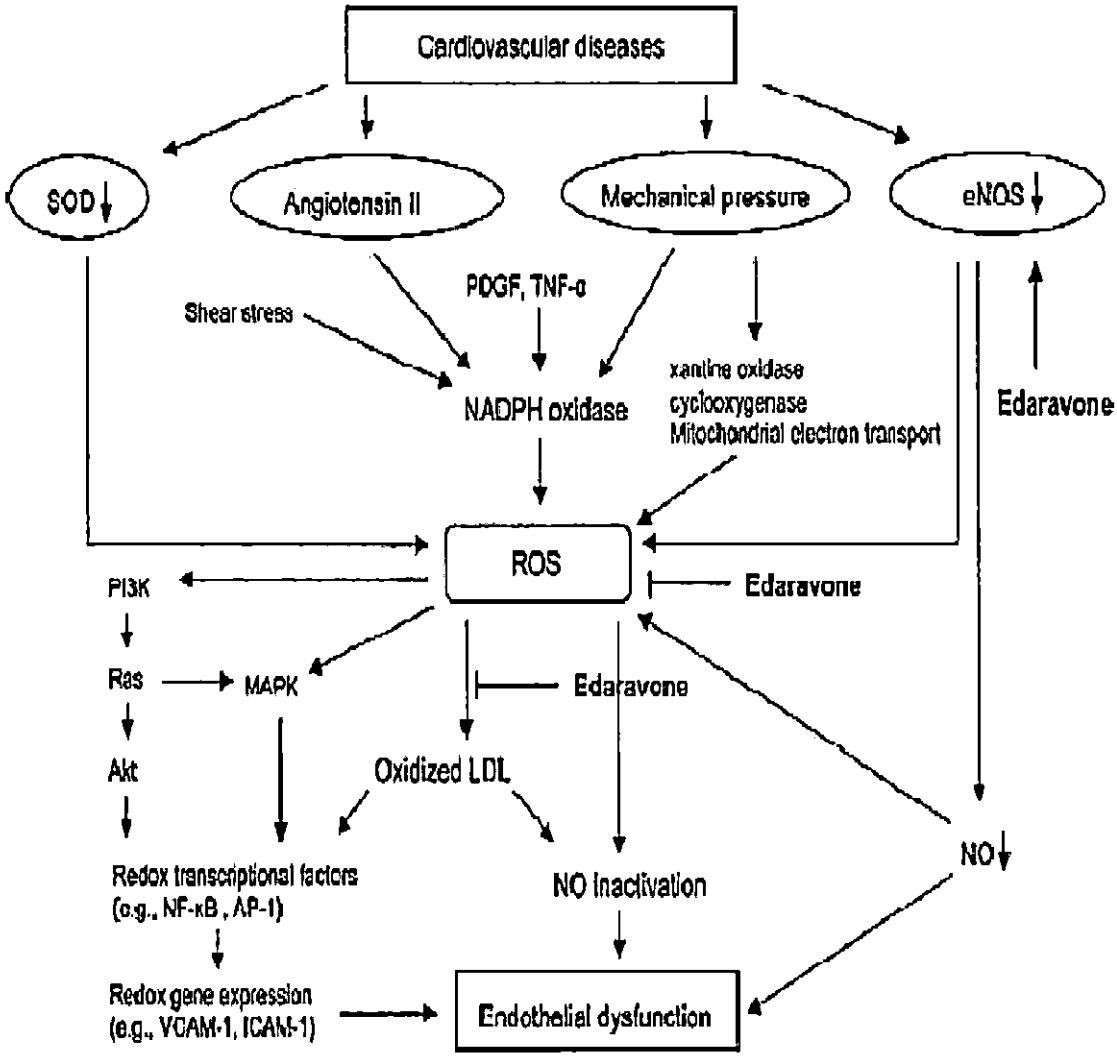

Fig.9. Putative mechanisms of edaravone-induced improvement in endothelial dysfunction in patients with cardiovascular disease

Pyrazolone derivatives reported to possess wide spectrum of activities. Recent research report shows that Pyrazolone could be useful in treatment of cholesterol ester storage disease and the treatment of conditions such as thrombosis and myocardial infarction, vasospastic disorders and bronchospasm or in reperfusion salvage therapy. But the literature and reported research articles have limited information on myocardial ischemic reperfusion injury.

2.11.4. Free radical scavenging activity

Recent research evidences that pyrazolones are reported as free radical scavangers. Pyrazolone derivatives, pertaining to the first groups of compounds such as Dipyrone aminopyrine, isopropylantipyrine and antipyrine used as analgesic, antipyretic, and anti-inflammatory therapeutic drugs (**Fig.10.**).

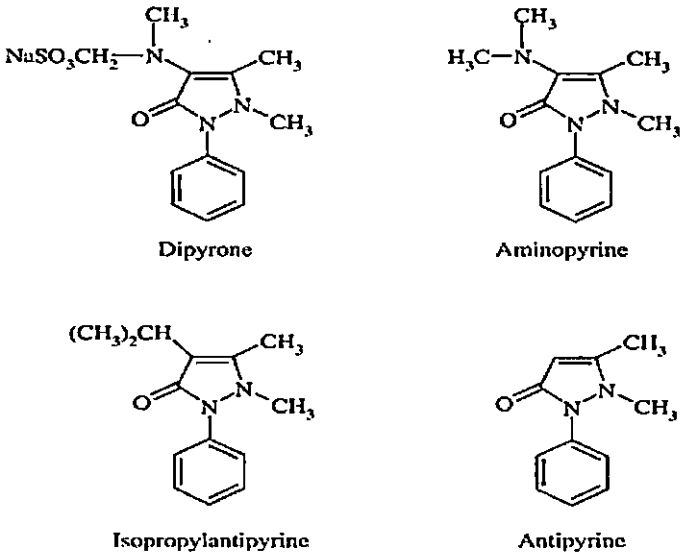

Fig.10. Structure of pyrazolone derivatives

The therapeutic activity of antipyrine was increased by an introduction of an isopropyl group on C-4 to produce isopropyl antipyrine, which improved the antipyretic and analgesic properties, while maintaining the anti-inflammatory activity. The introduction of a dimethylamino group on C-4 of the antipyrine molecule resulted in aminopyrine.

A disadvantage of aminopyrine is its relative insolubility in water. The search for more soluble compounds led to the production of the sodium salt of antipyridinyl methyl aminomethane sulfonic acid (dipyrone) (Brogden, 1986). The pharmacological mechanism of action of pyrazolones is that it involves the inhibition of cycloxygenase isoenzymes, platelet thromboxane synthesis, and prostanoids synthesis. The risk of agranulocytosis associated with the use of pyrazolone drugs at therapeutic doses and for short periods of time has been considered to be very low. However, little or no attention at all has been devoted to the possible hindrance of neutrophil burst and scavenging of neutrophil generated

reactive oxygen species (ROS) by these compounds. The studied pyrazolones was capable of scavenging O^{2-} or H_2O_2, while dipyrone was shown to be the most reactive against ROOS.

2.11.5. Role of pyrazolones for cerebrovascular diseases

Experimental studies have shown beneficial effects of edaravone on post ischemic reperfusion injury (Nakamura *et al.,* 2003; Takashi *et al.,* 2004; Nakajima *et al.,* 2005 ; Otani *et al.,* 2005 ; Ikeda *et al.,* 2002). Edaravone has been shown to ameliorate infarct size and brain edema in embolization and transient focal, global, and hemispheric ischemia models in adult rats (Takashi *et al.,* 2004., Jin *et al.,* 2002; Nakajima *et al.,* 2005) and to attenuate the hypoxic-ischemia encephalopathy in neonatal rats (Ikeda *et al.,* 2002).

In Japan, edaravone was approved in April 2001 for treatment of acute brain infarction and subarachnoid hemorrhage in the acute phase. Several investigators have reported that edaravone has beneficial effects on prevention of brain damage in patients with stroke (Ogasawara et al., 2004). Although the usefulness of edaravone for treatment of mild to moderate stroke in the acute phase has been established, it is unclear whether edaravone is effective against brain damage in patients with severe stroke.

2.11.6. TNF α and cytokine inhibition

The over expression of cytokines such as TNF-α and IL-16, has been implicated in a number of serious inflammatory disorders. Consequently, agents that inhibit the production of TNF- α can decrease levels of these pro-inflammatory cytokines and thereby reduce inflammation and prevent further tissue destruction in diseases such as rheumatoid arthritis (RA), osteoarthritis (OA), and Crohns disease. The efficacy of inhibitors against the overall cascade that leads to TNF-α production and have been identified p38 MAP kinase as one of the critical targets of inhibition.

Hence monocyclic and bicyclic pyrazolone derivatives have been synthesized and screened for cytokine inhibitory action. 4-Aryl-5-pyrimidyl based cytokine synthesis inhibitors that contain a novel monocyclic, pyrazolone heterocyclic core **(Fig.11.)**. One of the compounds was found to be efficacious in the rat iodoacetate (RIA) *in vivo* model of the pyrimidyl based cytokine synthesis inhibitors that contain a novel monocyclic, pyrazolone heterocyclic

core were described (Jennifer *et al.*, 2005) as cytokine synthesis inhibitors. Many other small molecules TNF- α production inhibitors have been reported containing a common 4-aryl- 5-pyrimidinyl based motif fused to a 5- or 6-membered heterocyclic core. A prototypical pyridyl imidazole-based inhibitor, although numerous structural classes, for example, pyrroles, pyrimidines, pyridines, pyrimidones, indoles, heteroindoles, ureas and various fused bicyclic heterocycles containing a variety of functionality have been reported to inhibit cytokine activity.

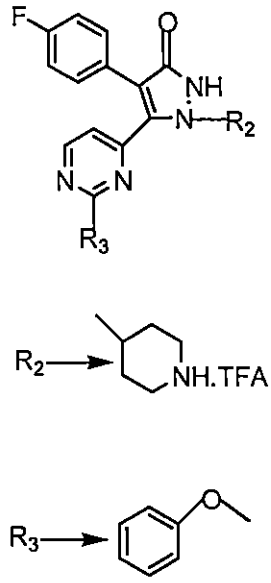

Fig.11. Structure of pyrimidine fused pyrazolones

2.11.7. Map kinase inhibition

Researchers from Merck reported studies on pyrazolones and other heterocycles as inhibitors of MAP p38 kinase (**Fig.12.**). Two monocyclic pyrazolones showed with good p38a-kinase inhibition, but weak whole cell assay activities. The alkyl substitution of both pyrazolone ring nitrogen atoms could facilitate cell membrane permeability and improve cellular cytokine synthesis inhibition profile.

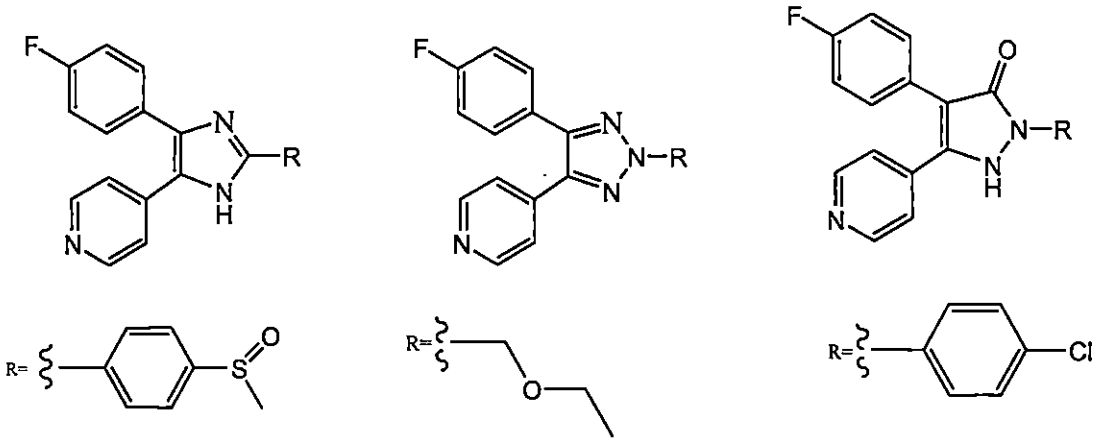

Fig.12. p38a-kinase/TNF -α synthesis inhibitors

All compounds were tested for the inhibition of TNF- α production using lipopolysaccharide stimulated human monocyclic cells based on a monocyclic pyrazolone scaffold at nanomolar activity in whole cell assay and reported to inhibit TNF- α.

2.12. CURRENT AND FUTURE DEVELOPMENTS ON CVS

The treatment of IHD involves expensive and chronic drug therapy or equally expensive interventional procedure such thrombolytic therapy and surgical recannulation, which has its own drawbacks in the form of reperfusion injury. The etiopathogenesis of this phenomenon is complex and multi-factorial of which oxygen free radical (OFR) has been identified as the major contributor. Efforts to control OFR induced damage, using modern pharmacological agent have met with little success. More importantly, the results of such will provide us with better cost effective and socially acceptable therapeutic options for a disease, which threatens to be the number one killer disease in this century.

The various drugs, which have so far been tried out are calcium antagonists, beta blockers and free radical scavenger. Although these drugs provide significant benefit in acute conditions and in secondary prevention, they are not advisable or acceptable for chronic use as primary preventive measures in large number of patients, who possess a high level of risk of having acute ischemic episodes, later in life. Moreover, chronic use of various drugs in the treatment of IHD showed major limitations due to various side effects. Under such circumstances, other options need to be explored which will help in circumventing this problem.

In this literature review, we indicated the possibility that pyrazolone has beneficial effects on not only myocardial and vascular injury following ischemia and reperfusion in patients with acute myocardial infarction, but also in atherosclerosis in the chronic phase. Due to the lack of clinical studies using pyrazolone it remains unclear whether pyrazolone treatment is beneficial for patients who have excess oxidative stress and whether pyrazolone reduces the mortality rate of these patients. It is expected that pyrazolone will be useful for treatment of various diseases in which oxidative stress may be involved in the pathogenesis. Awareness of the rising incidence of ischemic heart disease (IHD) in India, coupled with prohibitive cost of treatment, particularly for developing country generated urgency for the rapid development of an novel synthetic molecule with less side effects in the amelioration of IHD, hence the present study is aimed at evaluate the cardio protective effect of pyrazolone derivatives.

2.13. REFERENCES

- AIHW. Australia's health 2008. Cat. No. AUS 99. Canberra: AIHW.
- Allender S, Scarborough P, Flaherty MO, Capewell S. Patterns of coronary heart disease mortality over the 20th century in England and Wales: Possible plateaus in the rate of decline. BMC Public Health. 2008; 8:148.
- Avkiran M and Marber MS. Na^+/H^+ exchange inhibitors for cardioprotective therapy: progress, problems and prospects. *J Am Coll Cardiol.* 2002; 39: 747–753.
- Bahuleyan CG. Hospital data on coronary artery disease from North Kerala. In Vijayaraghavan G (ed). Cardiovascular Disease Prevention Trivandrum. pp. 54-59.
- Benjamin IJ, Schneider MD. Learning from failure: congestive heart failure in the postgenomic age. *J Clin Invest.* 2005; 115: 495-499.
- Bjork J, Hedqvist P, Arfors KE. Increase in vascular permeability induced by leukotriene B4 and the role of polymorphonuclear leukocytes. *Inflammation.* 1982; 6: 189-200.
- Bolli R. Mechanism of myocardial "stunning." *Circulation.* 1990; 82:723-738.
- Bolli R. The late phase of preconditioning. *Circ Res.* 2000; 87: 972–983.
- Brogden RN: Pyrazolone Derivatives, *Drugs.* 1986, 32(4), 60- 70.
- Buja LM. Myocardial ischemia and reperfusion injury. *Cardiovasc Pathol.* 2005; 14: 170-175.
- Carden DL, Granger DN. Pathophysiology of ischemia-reperfusion injury. *J Pathol.* 2000; 190: 255–266.
- Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res.* 2000; 87: 840-844.
- Chamoun F, Burne M, O'donnell M, Rabb H. Pathophysiologic role of selectins and their ligands in ischemia reperfusion injury. Frontiers in Bioscience 2000; 5: E103-9 mast cells. *Pharmacol Res.* 1997; 36: 2.

- Charles D, Collard M.D, Simon G. Pathophysiology, Clinical Manifestations, and Prevention of Ischemia–Reperfusion Injury. *Anesthesiology.* 2001; 94: 1133–1138.
- Chiari P, Bouvet F, Piriou V. Anaesthetic-induced myocardial preconditioning: fundamental basis and clinical implications. *Ann Fr Anesth Reanim.* 2005; 24: 383-396.
- Chien KR. Stress pathways and heart failure. *Cell* 1999; 98: 555-8. *Circulation.* 1993; 87: 152-161.
- Collard CD, Park KA, Montalto MC, Alapati S, Buras JA, Stahl GL, Colgan SP. Neutrophil-derived glutamate regulates vascular endothelial barrier function. *J Biol Chem.* 2002; 277: 14801-14811.
- Dwivedi S. Atherosclerosis revisited. *Indian J Cardiol.* 2004; 7: 6-12.
- Delles C, Schneider MP, John S, Gekle M, Schmieder RE. Angiotensin converting enzyme inhibition and angiotensin II AT1-receptor blockade reduce the levels of asymmetrical N (G), N (G)-dimethylarginine in human essential hypertension. *Am J Hypertens.* 2002; 15: 590-593.
- Eltzschig HK, Ibla JC, Furuta GT, Leonard MO, Jacobson KA, Enjyoji K, Robson SC, Colgan SP. Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in post hypoxic endothelium: role of ectonucleotidases and adenosine A2B receptors. *J Exp Med.* 2003; 198: 783-796.
- Ferrari R and Visioli O. Particular outcomes of myocardial ischemia: Stunning and hibernation. *Pharmacol Res.* 1995; 31: 235-241.
- Flaherty OM, Ford E, Allender S, Scarborough P, Capewell S. Coronary heart diseasetrends in England and Wales from 1984 to 2004: concealed levelling of mortality rates among young adults. *Heart.* 2008; 94:178–181.
- Ford E, Capewell S. Coronary heart disease among young adults in the U.S. from 1980 to 2002. Concealed levelling of mortality rates. *J Am Coll Cardiol.* 2007; 50:2128–2132.

- Fujita MSH, Wakabayashi Y, Morita I. Cell adhesion molecule mediates endothelial cell injury caused by activated neutrophils. *Keio Journal of Medicine.* 1996; 45: 207-212.
- Fukai T, Siegfried MR, Ushio-Fukai M, Cheng Y, Kojda G, Harrison DG. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. *J Clin Invest.* 2000; 105: 1631-1639.
- Girija G. Risk factors profile of patients with acute MI. In Vijayaraghavan G (ed). Cardiovascular Disease Prevention Trivandrum. 78-83. American Heart Association Heart and Stroke Statistical Update. 1997; 26-27.
- Goel PK, Bharti BB, Pandey CM, et al. A tertiary care hospital-based study of conventional risk factors including lipid profile in proven coronary artery disease. *Indian Heart J.* 2003; 55:234-240.
- Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part II: animal and human studies.*Circulation.* 2003; 108: 2034-2040.
- Gross ER, Gross GJ. Ligand triggers of classical preconditioning and postconditioning. *Cardiovas Res.* 2006; 70: 212-221.
- Heusch G, Schulz R. Remote preconditioning. *J Mol Cell Cardiol.* 2002; 34: 1279-1281.
- Hoogeveen RC, Gambhir JK, Gambhir DS, et al. Evaluation of Lp[a] and other independent risk factors for CHD in Asian Indians and their USA counterparts. *J Lipid Res.* 2001; 42:631-638.
- Hughes K, Lun KC, Yeo PPB. Cardiovascular disease in Chinese Malays and Indian in Singapore: Difference in mortality. *J Epidemiol Community Health.* 1990; 44: 24-28.
- Ikeda T, Xia YX, Kaneko M, Sameshima H, Ikenoue T. Effect of the free radical scavenger, 3-methyl-1-phenyl-2-pyrazolin-5-one (MCI-186), on hypoxia-ischemia-induced brain injury in neonatal rats. *Neurosci Lett.* 2002; 329: 33-36.

- Jenkins DP, Steare SE, Yellon DM. Preconditioning the human myocardium: recent advances and aspirations for the development of a new means of cardioprotection in clinical practice. *Cardiovasc Drugs Ther.* 1995; 9: 739-747.
- Jennifer A, Matthew J, Laufersweiler, Todd AB. Michael PC, Cynthia MC. The development of monocyclic pyrazolone based cytokine synthesis inhibitors. *Bioorg Med Chem Lett.* 2005; 15: 2285–2289.
- Kevelaitis E, Oubenaissa A, Mouas C, Peynet J, Menasche P. Ischemic preconditioning with opening of mitochondrial adenosine triphosphate sensitive potassium channels or Na/H exchange inhibition: which is the best protective strategy for heart transplants? *J Thorac Cardiovasc Surg.* 2001; 121: 155-162.
- Kloner RA, Rezkalla SH. Preconditioning, postconditioning and their application to clinical cardiology. *Cardiovas Res.* 2007; 70: 297-307.
- Kono H, Asakawa M, Fujii H, et al. Edaravone, a novel free radical scavenger, prevents liver injury and mortality in rats administered endotoxin. *J Pharmacol Exp Ther.* 2003; 307: 74-82.
- Luscinskas FW, Ma S, Nusrat A, Parkos CA, Shaw SK. Leukocyte transendothelial migration: a junctional affair. *Semin Immunol.* 2002; 14: 105-113.
- Mammi MVI, Pavithran P, Rahman PA et al. Acute MI in North Kerala. A 20-year hospital based study. *Indian Heart J.* 1991; 43: 93-96.
- Masaki Y, Kumano K, He N, Suyama I, Endo T. Protective effects of MCI-186 on cold kidney preservation/reperfusion injury in the rat. *Transplant Proc.* 1996; 28: 1885-1886.
- Maxwell SR, Lipp GY. Reperfusion injury: A review of the pathophysiology, clinical manifestations and therapeutic options. *Int J Cardiol.* 1997; 58: 95-117.
- Mc Clahan TB, Nao, BS, Wolke LJ, Martin BS, Metz TE and Gallagher, KP. Brief renal occlusion and reperfusion reduces myocardial infarct size in rabbits. FASEB 1993; 7: A118-23.

- McDonough JL, Arrell DK, Van Eyk JE. Troponin I. Degradation and Covalent Complex Formation Accompanies Myocardial Ischemia/Reperfusion Injury. *Circ Res.*1999; 84: 9-20.
- Minhaz U, Tanaka M, Tsukamoto H. Effect of MCI-186 on postischemic reperfusion injury in isolated rat heart. *Free Radic Res.* 1996; 24: 361-7.
- Morrison RR, Xing Lin Tan,Catherine Ledent, S. Jamal Mustafa, and Polly A. Hofmann Targeted deletion of A2A adenosine receptors attenuates the protective effects of myocardial postconditioning. *Am J Physiol Heart Circ Physiol.* 2007; 293: H2523-9.
- Muller V, losonczy G, vannay U, Fekete A, reusz G, Tulassay T and Szabo AJ. Sexual dimorphism in renal ischemia-reperfusion injury in rats: Possible role of endothelin. *Kidney International.* 2002; 62: 1364-1371.
- Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation.* 1986; 74: 1124–1136.
- Nakajima H, Kakui N, Ohkuma K, Ishikawa M, Hasegawa T. A newly synthesized poly (ADP-ribose) polymerase inhibitor, DR2313 [2-methyl-3,5,7,8-tetrahydrothiopyrano[4,3-d]-pyrimidine-4-one]: Pharmacological profiles, neuroprotective effects, and therapeutic time window in cerebral ischemia in rats. *J Pharmacol Exp Ther.* 2005; 312: 472-481.
- Nakamura H, Uzura M, Uchida K, et al. Effects of edaravone on experimental brain injury in view of free radical reaction. *Acta Neurochir Suppl.* 2003; 86: 309-311.
- Negus BH, Williard JE, Glamann DB et al. Coronary anatomy and prognosis of young asymptomatic survivors of myocardial infarction. *Am J Med.* 1994; 96: 354-358.
- Ogasawara K, Inoue T, Kobayashi M, Endo H, Fukuda T, Ogawa A. Pretreatment with the free radical scavenger edaravone prevents cerebral hyperperfusion after carotid endarterectomy. *Neurosurgery.* 2004; 55: 1060-1067.
- Oliver CN, Starke-reed PE, Stadtman ER, Lidt GJ, Carneyt JM, and Floydt RA. Oxidative damage to brain proteins, loss of glutamine synthetase activity, and

production of free radicals during ischemia/ reperfusion-induced injury to gerbil brain. Neurobiology 1990; 87: 5144-5147.

- Ono H, Osanai T, Ishizaka H, Hanada H, Kamada T, Onodera H, Fujita N, Sasaki S, Matsunaga T, Okumura K. Nicorandil im-proves cardiac function and clinical outcome in patients with acute myocardial infarction undergoing primary percutaneous coronary intervention: role of inhibitory effect on reactive oxy-gen species formation. *Am Heart J.* 2004; 148: E15.
- Otani H, Togashi H, Jesmin S, et al. Temporal effects of edaravone, a free radical scavenger, on transient ischemia-induced neuronal dysfunction in the rat hippocampus. *Eur J Pharmacol.* 2005; 512: 129-137.
- Patil SS, Joshi R, Gupta G, et al. Risk factors for acute myocardial infarction in a rural population of central India: a hospital-based case-control study. *Natl Med J India.* 2004; 17:189-194.
- Pell TJ, Baxter GF, Yellon DM, Drew GM. Renal ischemia preconditioning myocardium role of adenosine receptors and ATP-sensitive potassium channels. *Am J Physiol.* 1998; 275: H1542-7.
- Post H, Heusch G. Ischemic preconditioning. Experimental facts and clinical perspective. *Minerva Cardioangiol.* 2002; 50: 569-605.
- Przyklenk K, Baurer B, Ovize M, Kloner RA and Whittaker P. Regional ischemic "preconditioning" protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation.* 1993; 87: 893-899.
- Przyklenk K, Darling CE, Dickson EW, Whittaker P. Cardioprotection 'outside the box' the evolving paradigm of remote preconditioning. *Basic Res Cardiol.* 2003; 98: 149-157.
- Quintana M, Kahan T, Hjemdahl P. Pharmacological prevention of reperfusion injury in acute myocardial infarction. A potential role for adenosine as a therapeutic agent. *Am J Cardiovasc Drugs.* 2004; 4: 159–167.
- Rajadurai J, Arokiasami J, Pasamanichan K et al. Amei Lin O. Coronary disease in Asians. *Aus Nz J Med.* 1992; 22: 345-348.

- Ramzy D, Rao V, Weisel RD. Clinical applicability of preconditioning and postconditioning: The cardiothoracic surgeons's view. *Cardiovas Res.* 2007; 70: 174-180
- Ress DD, Palmer RMJ, Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA.* 1989; 86: 3375-3378.
- Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation.* 2000; 101: 1899-1906.
- Schoemaker RG, Van Heijuingan CL. Bradykinin mediates cardiac preconditioning at a distance. *Circulation.* 2000; 278: H1571-6.
- Schulman D, Latchman DS, Yellon DM. Effect of aging on the ability of preconditioning to protect rat hearts from ischemia–reperfusion injury. *Am J Physiol Heart Circ Physiol.* 2001; 281: H1630-6.
- Schwartz LM, Lagranha CJ. Ischemic postconditioning during reperfusion activates Akt and ERK without protecting against lethal myocardial ischemia-reperfusion injury in pigs. *Am J Physiol Heart Circ Physiol.* 2006; 290: H1011-8.
- Singh M, Sharma A. Mechanism of cardioprotective effect of remote aortic preconditioning. In pathophysiology of cardiovascular diseases.2004; Dhalla NS, Angel RA and Pierce, GN (eds) Kluswer Academic Publishers, Boston, 275-285.
- Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, Aupetit JF, Bonnefoy E, Finet G, André-Fouët X, Ovize M. Postconditioning the human heart. *Circulation.* 2005; 112: 2143-2148.
- Stocker R, Keaney JF Jr. Role of oxidative modifications in atherosclerosis. *Physiol Rev.* 2004; 84: 1381-1478.
- Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr., Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation.* 2000; 101: 948-954.

- Takahashi G, Sakurai M, Abe K, Itoyama Y, Tabayashi K. MCI- 186 reduces oxidative cellular damage and increases DNA repair function in the rabbit spinal cord after transient ischemia. *Ann Thorac Surg.* 2004; 78: 602-607.
- Teoh LK, Grant R, Hulf JA, Pugsley WB, Yellon DM. The effect of preconditioning [ischemic and pharmacological] on myocardial necrosis following coronary artery bypass graft surgery. *Cardiovasc Res.* 2002; 53: 175–180.
- The Lancet (2008). 371[9622]: pp. 1435 - 1442.
- Tosaka M, Hashiba Y, Saito N, Imai H, Shimizu T, Sasaki T. Contractile responses to reactive oxygen species in the canine basilar artery in vitro: selective inhibitory effect of MCI-186, new hydroxyl radical scavenger. *Acta Neurochir.* 2002; 144: 1305-1310.
- Tsang A, Hausenloy DJ, Mocanu MM, Carr RD, Yellon DM. Preconditioning the diabetic heart: the importance of Akt phosphorylation. *Diabetes.* 2005; 54: 2360-2364.
- Tsang A, Hausenloy DJ, Mocanu MM, Yellon DM. Postconditioning: a form of "modified reperfusion" protects the myocardium by activating the phosphatidylinositol 3 kinase-Akt path-way. *Circ Res.* 2004; 95: 230-232.
- Verma S, Paul WM, Fedak RD. Weisel MD. Butany J, Rao V, Maitland A, Li RK, Dhillon B, Yau TM. Fundamentals of Reperfusion Injury for the Clinical Cardiologist. *Circulation.* 2002; 105: 2332-2336.
- Vinten-Johansen J, Zhao ZQ, Jiang R, Zatta AJ, Dobson GP. Preconditioning and postconditioning: innate cardioprotection from ischemia-reperfusion injury. *J Appl Physiol.* 2007; 103: 1441-1448.
- Wang Y, Xu H, Mizogychi K, Oe M, Mata H. Intestinal ischemia induces rate preconditioning against myocardial infarction. A role of inducible nitric oxide synthase. *Cardiovasc Res.* 2001; 49, 391-398.
- Weinbrenner C, Nelles M, Herzog, N, Sarvary L, Strasser RH. Remote preconditioning by intrarenal occlusion of the aorta protects the heart from infarction: a newly identified non-neuronal but PKC-dependent pathway. *Cardiovasc Res.* 2002; 55: 590-601.

- Weiss SJ: Tissue destruction by neutrophils. *New Engl J Med.* 1989; 320: 365-376.
- WHO, 2005.Fact sheet about cardiovascular disease 2005, world health organisational , Regional Office for SE Asia, New Delhi.
- Yamaguchi T, Oishi K, Uchida M, Echizen H. Edaravone, a radical scavenger, may enhance or produce antiproliferative effects of fluvastatin, amlodipine, ozagrel, GF109203X and Y27632 on cultured basilar artery smooth muscle cells. *Biol Pharm Bull.* 2003; 26: 1706-1710.
- Yamamoto Y, Kuwahara T, Watanabe K, Antioxidant activity of 3-methyl-1-pheneyl-2-pyrazolin-5-one. *Redox Report.* 1996; 2: 333-338.
- Yamawaki M, Sasaki N, Shimoyama M, et al. Protective effect of edaravone against hypoxia-reoxygenation injury in rabbit cardiomyocytes. *Br J Pharmacol.* 2004; 142: 618-626
- Yanagisawa A, Miyagawa M, Ishikawa K, Murota S. Cardioprotective effect of MCI-186 (3-methyl-1-phenyl-2- pyrazolin-5-one) during acute ischemia-reperfusion injury in rats. *Int J Angiology.* 1994; 3: 12-15.
- Yang XM, Krieg T, Cui L, Downey JM, Cohen MV. NECA and bradykinin at reperfusion reduce infarction in rabbit hearts by signaling through PI3K, ERK, and NO. *J Mol Cell Cardiol.* 2004; 36: 411-421.
- Yellon DM and Hausenloy DJ. Realizing the clinical potential of ischemic preconditioning and postconditioning. *Nature.* 2005; 2: 11.
- Yellon DM, Baxter GF. A "second window of protection" or delayed preconditioning phenomenon: future horizons for myocardial protection? *J Mol Cell Cardiol.* 1995; 27: 1023-1034.
- Yoshida H, Sasaki K, Namiki Y, Sato N, Tada N. Edaravone, a novel radical scavenger, inhibits oxidative modification of lowdensity lipoprotein (LDL) and reverses oxidized LDL-mediated reduction in the expression of endothelial nitric oxide synthase. *Atherosclerosis.* 2005; 179: 97-102.

- Zhang XH, Matsuda N, Jesmin S, et al. Normalization by edaravone, a free radical scavenger, of irradiation-reduced endothelial nitric oxide synthase expression. *Eur J Pharmacol.* 2003; 476: 131-137.
- Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Corvera ME, Kerendi F, Wang NP, Guyton RA, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am *J Physiol Heart Circ Physiol.* 2003; 285: H579-588.
- Zhao ZQ, Vinten-Johansen J. Postconditioning: Reduction of reperfusion-induced injury. *Cardiovasc Res.* 2006; 70: 200-211.